

Characterization and localization of leptin receptors in the rat kidney

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Abstract Characterization and localization of leptin binding sites were investigated in rat kidneys using [125 I]leptin as a ligand. [125 I]leptin specific binding was found in high amounts in rat renomedullary membranes. This binding was specific, saturable, time-dependent ($K_{\text{obs}} = 0.055 \pm 0.008 \text{ min}^{-1}$) and the dissociation of receptor-bound ligand was slowly reversible ($K_{-1} = 0.048 \pm 0.013 \text{ min}^{-1}$). From saturation experiments, a single class of high-affinity binding sites for leptin was identified with an apparent K_d of $0.57 \pm 0.14 \text{ nM}$ and a B_{max} of $45 \pm 10 \text{ fmol/mg protein}$. [125 I]leptin binding was inhibited in a dose-dependent manner by cold leptin and was highly selective since not displaceable by a number of other hormones or peptides. Autoradiographic experiments performed on adult rat kidney sections showed the intense presence of [125 I]leptin receptors only in specific areas of the renal inner medulla and also consistent labeling associated with vascular structures in the cortico-medullary region. The study of the postnatal developmental expression of leptin receptors in the kidney showed very low expression during the early postnatal period (8–21 days). Full expression of leptin sites was achieved at about 30 days and remained stable throughout adulthood (60 days and upwards). Moreover, *in vivo* administration of leptin (0.5 mg/kg *i.p.*) induced a significant and rapid diuretic effect in normally hydrated conscious rats. Thus, these data constitute the first characterization and mapping of [125 I]leptin specific binding sites in the rat kidney and raise the possibility of a renal control by leptin.

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Key words: Leptin; Kidney; Rat; Binding; Autoradiography

1. Introduction

Leptin, the *ob*-gene product, is a recently isolated 16 kDa hormone highly conserved among vertebrates, exclusively synthesized and secreted by adipose tissue into the blood stream [1–4]. In obese leptin-deficient *ob/ob* mice, this hormone was initially identified as a key satiety signal able to reduce food intake, fat tissue, hyperglycemia and hyperinsulinemia presumably by interaction with specific receptors expressed at a high level in the hypothalamus [5]. Thus, this factor was expected to be a central regulator in controlling body weight and treating obesity. However, with the exception of the *ob/ob* mice strain, circulating leptin plasma levels are in general elevated in obesity, suggesting that leptin resistance rather than leptin deficiency seems a common feature of obesity in human [6–8], primate [4] and in most models of rodent obesity [9]. In fact, it is a defect in the leptin receptor which causes obesity. The leptin receptor has been cloned and up to now, various alternatively spliced forms of the leptin receptor

mRNA (at least 6 in mice) have been discovered in several species including mice, rat and human [10–14].

While most attention of the biological effects of leptin have been focused on the hypothalamus, the mRNA of several leptin receptor isoforms—including the leptin receptor long transcript, identified as the functional receptor—has also been found, with a different pattern of distribution, in a number of non-neuronal tissues such as the lung, liver, kidney, heart, reproductive organs and fat [10–14]. These preliminary findings could implicate leptin in pathways other than energy metabolism and suggest that leptin receptors, when expressed, could mediate a variety of direct peripheral actions.

In an attempt to determine the role of leptin in the regulation of the renal function, the purpose of the present study was to investigate the expression, characterization and distribution of leptin receptors in the rat kidney using [125 I]leptin as a ligand. By autoradiographic techniques, the developmental expression of leptin receptors was also examined to gain information on the role of this hormone in renal cellular growth and organ development. Finally, the functional role of leptin in the kidney was further investigated by studying its effect on diuresis after *in vivo* administration in rats.

2. Materials and methods

2.1. Materials

2.1.1. Chemicals. Recombinant murine leptin was obtained from Pepro Tech Inc. (Rocky Hill, NJ). Bacitracin, leukemia inhibitory factor (LIF), insulin, interleukin-6 (IL-6), granulocyte-colony stimulating factor (G-CSF), and various peptides were from Sigma Chemical Co. (L'isle d'Abeau, France). Bovine serum albumin was obtained from Biosepra (Paris, France). All other chemicals were from Merck-Clevenot (Nogent sur Marne, France) or Prolabo (Paris, France).

The [125 I]murine leptin (2000 Ci/mmol) was kindly provided by New England Nuclear (Les Ulis, France).

2.1.2. Animals. Male Sprague-Dawley rats were obtained at different ages (8, 21, 30, 60 and 84 days; b.wt. 18, 50, 90, 280 and 420 g, respectively) from Charles River Breeding Laboratories, Inc. (Saint-Aubin les Elbeuf, France). The animals were housed in a temperature-, humidity- and light-controlled room (12-h light/dark cycle) and allowed free access to water and standard rat chow. All protocols performed in this study have been approved by the Animal Care and Use Committee of Sanofi Recherche.

2.2. Membrane preparations

Male Sprague-Dawley rats (60 days old, b.wt. 250–300 g) were killed by decapitation and the kidneys were immediately removed. The two anatomic structures, cortex and medulla (i.e. inner–outer medulla and papilla) were rapidly dissected and collected in ice-cold saline, 1 mM EDTA, pH 7.5, for membrane preparations. Membranes resulting from each structure were prepared by the method of Stassen et al. [15] and stored as aliquots in liquid nitrogen (5 mg protein/ml). Protein concentration was determined by the method of Bradford using bovine serum albumin as a standard [16].

2.3. Binding assays

Rat kidney membranes (50–100 $\mu\text{g/ml}$) were incubated for 90 min at 20°C in a 200 μl of Krebs-Ringer medium (pH 7.4) containing 20

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mM HEPES, 1% (w/v) bovine serum albumin, 300 µg/ml bacitracin and 60 pM [125 I]leptin. The incubation was stopped by adding 4 ml of ice-cold buffer and the contents of the assay tubes were filtered rapidly through Whatman GF/C filters, then rinsed with 8 ml of ice-cold wash buffer. The radioactivity of the filter was counted in an LKB multiwell gamma counter. Non-specific binding was determined by incubating with 0.3 µM cold leptin. Competition studies were performed on medullary membranes with 60 pM [125 I]leptin in the presence of increasing concentrations of unlabeled leptin (0.003, 0.01, 0.03, 0.1, 0.3, 0.6, 1, 3, 6, 10, 30, and 100) or of various concentrations of peptides or hormones. The association constant was determined by measuring the binding of 60 pM [125 I]leptin to the medullary membranes at different times (3, 6, 9, 12, 15, 20, 30, 45, 60, 75, 90, 120, 150, 180, 240, 270, and 330 min) and dissociation was initiated at 90 min by adding 1 µM unlabeled leptin.

The IC_{50} value was defined as the concentration of inhibitor required to obtain 50% inhibition of the specific binding. Inhibition constant (K_i) values were calculated from the IC_{50} values using the Cheng and Prusoff equation [17]. Data for equilibrium binding [apparent equilibrium dissociation constant (K_d), maximum binding density (B_{max})], competition experiment [IC_{50} , Hill coefficient (n_H)], and kinetic constants [apparent association rate constant (K_{obs}), apparent dissociation rate constant (K_{-1})], were analyzed using an interactive non-linear regression program [18].

2.4. Autoradiography

Male Sprague-Dawley rats at various ages (8, 21, 30, 60 and 84 days old) were decapitated and the kidneys were rapidly removed and frozen in isopentane at $-40^{\circ}C$. Serial sections (16 µm) were cut in a cryostat microtome and thaw-mounted into chrom-alum gelatin-coated glass slides (gelatin 1%, chrom-alum 0.05%). Sections were stored at $-80^{\circ}C$ until use. Slide-mounted sections (3–10 sections/slide according to the size of the kidney), brought to room temperature, were preincubated for 15 min in the binding buffer (Krebs-Ringer medium; pH 7.4, containing 20 mM HEPES, 1% (w/v) bovine serum albumin, 300 µg/ml bacitracin). Incubation was carried out for 90 min at room temperature in the incubating medium in the presence of about 40 pM [125 I]leptin. Non-specific binding was determined by incubating additional adjacent rat kidney sections under the same conditions and in the presence of 0.3 µM unlabeled leptin. After incubation, the slides were washed 3 times for 10 min each in ice-cold buffer, dipped briefly in distilled water, and dried for 60 min under a stream of cold air [19,20]. The slide-mounted sections were exposed to X-ray Hyperfilm- 3H (Amersham, Les Ulis, France) for 7 days at $-20^{\circ}C$ to generate autoradiographic images.

2.5. Diuretic activity of leptin in conscious rats in vivo

Leptin (0.5 or 1 mg/kg) was administered intraperitoneally (1 ml/kg) to conscious normally hydrated or to water-loaded (25 ml bidistilled water/kg by gavage just before starting the experiment) rats (250 g b.wt.). The animals were then housed individually in metabolic cages without food and water and urines were collected for 6 h with individual measures of urine volume at 0.5, 1, 2, 4 and 6 h. The effect of leptin on urine osmolality was studied at each 2-h period for 6 h following leptin or vehicle administration. Urine osmolality was measured with a freezing point depression osmometer (model M85501, Roebling, Bioblock Scientific, France).

Control rats received the same volume of bidistilled water. Urine volume of the control and treated groups was compared using the non-parametric test of Mann-Whitney corrected by Bonferroni using RS/1 software. Statistical significance of leptin effect on miction latency was analyzed according to a Kruskal-Wallis' test using StatxAct software. The level of significance was taken as $P < 0.05$. Results are means $\pm$ SEM.

3. Results

3.1. Characterization of [125 I]leptin binding sites in rat kidney membranes

In preliminary experiments, binding sites of [125 I]leptin were studied in renal membrane preparations resulting from two main anatomic structures of the adult rat kidney, the cortex and the medulla. Specific binding was almost undetectable in

cortical preparations whereas significant amounts of specific labeling were found in medullary membranes as a function of the protein content in the assay (not shown). The time-course of association (Fig. 1) showed that specific binding of [125 I]leptin to rat kidney medullary membranes was time-dependent ($t_{1/2} = 15 \pm 5$; $n = 3$) and reached an apparent equilibrium in about 60 min ($K_{obs} = 0.055 \pm 0.008 \text{ min}^{-1}$). After 90 min of incubation, the binding of [125 I]leptin was reversed by the addition of unlabeled leptin (1 µM) to the assay. Dissociation of the leptin–receptor complex (Fig. 1) occurred slowly with an apparent dissociation constant (K_{-1}) value of $0.048 \pm 0.012 \text{ min}^{-1}$. However, the process seemed to be bi-phasic since after 5.5 h in the presence of 1 µM cold leptin, the dissociation was not total and reached 80%. From the kinetic experiments, a calculated K_d value of 0.5 nM was obtained. After equilibrium, under standard conditions, non-specific binding represented about 30% of total binding.

Saturation experiments indicated that the specific binding was saturable (Fig. 2A). Scatchard analysis of these data (Fig. 2B) gave a linear plot consistent with the presence of a single class of high-affinity binding sites characterized by an apparent K_d of $0.57 \pm 0.14 \text{ nM}$ and a maximal binding capacity (B_{max}) of $43 \pm 10 \text{ fmol/mg protein}$ ($n = 4$).

As shown in Fig. 3, [125 I]leptin binding was inhibited in a dose-dependent manner by unlabeled leptin with an IC_{50} value of $0.72 \pm 0.19 \text{ nM}$ ($n = 5$) and a pseudo Hill coefficient (n_H) about unit ($n_H = 0.99 \pm 0.15$, $n = 5$) consistent with competitive antagonism. It was interesting to note that the K_d value obtained from Scatchard plot was consistent with those obtained from kinetic and competition experiments.

Moreover, binding of [125 I]leptin was highly specific since various seven transmembrane G-protein-coupled receptor peptides (neuropeptide Y, peptide YY, pancreatic polypeptide, cholecystokinin, endothelin, neurotensin, corticotropin releasing factor, vasopressin, oxytocin, 1 µM) and several closely related cytokine receptor factors (0.2 µg/ml IL-6, 0.5 µg/ml leukemia inhibitory factor, 100 µM insulin and 0.25 µg/ml

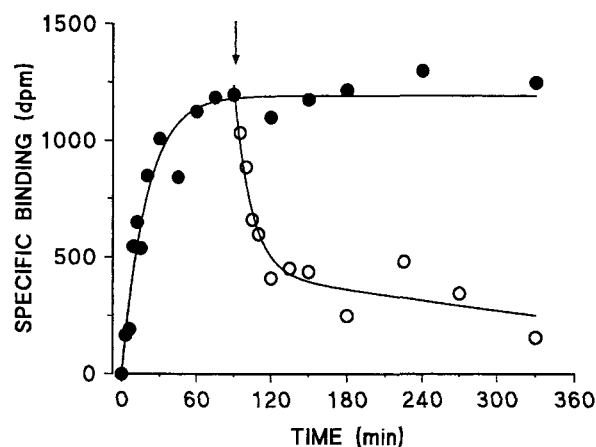


Fig. 1. Time-course of association (●) and dissociation (○) of [125 I]leptin to rat kidney medullary membranes. Incubations were carried out as described in Section 2 in the presence of 55 pM [125 I]leptin for various periods of time. Arrow indicates time (90 min) at which cold leptin (1 µM) was added to initiate the dissociation process. Results represent data from a typical experiment performed in duplicate and repeated 3 times without noticeable modifications.

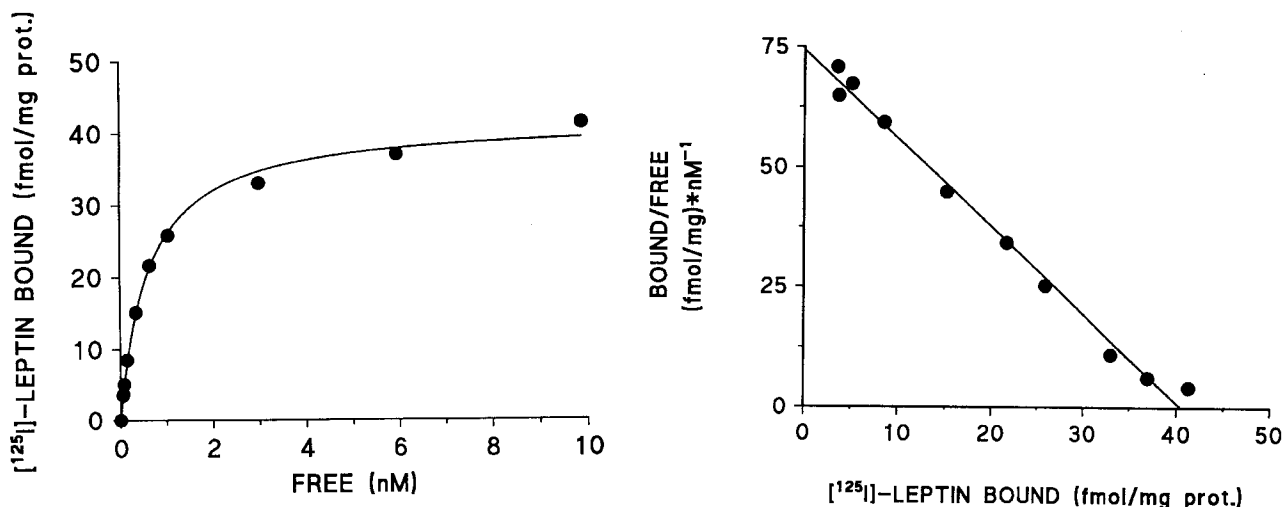


Fig. 2. Saturation of $[^{125}\text{I}]$ leptin specific binding to rat kidney medullary membranes. A: Saturation isotherm; B: Scatchard plot. Data are means calculated from a typical experiment performed in duplicate and repeated 4 times without noticeable changes.

ml granulocyte-colony stimulating factor) were unable to inhibit $[^{125}\text{I}]$ leptin specific binding to rat kidney membranes.

3.2. Autoradiographic distribution of leptin binding sites in the rat kidney

In the adult (60 days old) rat kidney, $[^{125}\text{I}]$ leptin intensely labeled the medullary region whereas an absence of labeling was observed in renal cortex, outer stripe and extremity of papilla (Fig. 4A) showing that no binding sites were evidenced on renal structures such as the glomeruli, proximal and distal tubules, and terminal collecting ducts.

In the medullary area two types of labeling were shown. We observed massive central labeling with $[^{125}\text{I}]$ leptin in the inner medulla region which spread from inner medulla to inner stripe of outer medulla as radiating streaks. This pattern is consistent with labeling associated with vascular structures such as vasa recta and/or descending thin Henle's limbs belonging to both short and long looped-nephrons. $[^{125}\text{I}]$ leptin showed also consistent binding to the renal pelvic wall, an epithelium structure which delineates the papillary surface and which corresponds to a remnant of capsula fibrosa.

Finally, it is important to note sparse individual spots of $[^{125}\text{I}]$ leptin localized in the renal cortico-medullary area, probably corresponding to the wall of big blood vessels cut in this region which could be an association of arterial and vein as observed under microscope (not shown). The association of $[^{125}\text{I}]$ leptin to vessels was clearly evidenced in autoradiograms performed in transversal rat kidney sections at the level of the medullary region (Fig. 4B).

3.3. Renal developmental expression of $[^{125}\text{I}]$ leptin binding sites

Expression of $[^{125}\text{I}]$ leptin binding sites was visualized in the kidney of developing (8–30 days old) and adult (60 and 84 days old) rats by autoradiography (Fig. 5). In contrast to a number of other organs, the kidney is not morphologically and functionally fully differentiated until about day 60, referred to the adult period [21,22]. Although the autoradiographic techniques used did not allow localization at the cellular level, this study provided further evidence that

$[^{125}\text{I}]$ leptin labeling was firstly age-dependent and secondly was mainly associated with medulla from the 21–30-day post-natal period. The labeling was emphasized in this latter anatomic structure at adult stages (Fig. 5C,D). At the earliest developmental stage investigated, 8 days, specific $[^{125}\text{I}]$ leptin labeling was almost undetectable in the kidney except in a discrete area of the medullary region (Fig. 5A). There was also little expression of leptin binding sites in the 21-day-old rat kidney; however, a general mapping of the medullary region appeared at this stage (Fig. 5B). The density of leptin binding sites to individual anatomic structures of the kidney was markedly increased between 21 and 30 days (Fig. 5C) and was concentrated in the central medulla. This mapping remained stable through adulthood and has a similar profile to that observed at 60 days (Fig. 4) and up to 84 days (Fig. 5D).

3.4. Effect of leptin on diuresis in conscious Sprague-Dawley rats

A preliminary set of experiments was performed in water-loaded rats to investigate potential antidiuretic properties for leptin. In this model, vasopressin levels are presumably very low [23] and diuresis sufficiently high to detect any antidiuretic component. As shown in Table 1, leptin (1 mg/kg i.p.) had no influence on urine volume within the 6-h observation period and no potential antidiuretic action was detected.

On the other hand, as shown in Fig. 6, acute administration of leptin (0.5 mg/kg i.p.) significantly increased urine excretion during the first-hour collection period in normally hydrated conscious rats. The time-course study showed a consistent and rapid diuretic effect of leptin on urine flow rate that was highly significant at 0.5 h. This effect disappeared within 2 h after the administration of leptin. As a consequence of the diuretic effect obtained, a significant ($P < 0.001$) decrease in urine excretion latency was observed for the leptin-treated group (i.e. 60% of the leptin-treated animals had their first miction at 0.5 h whereas the same proportion was reached after 3 h in the control group). Leptin also lowered significantly ($P < 0.05$) urine osmolality only in the first 2-h period: 757 ± 50 mOsm/kg H_2O ($n = 7$) and 479 ± 53 mOsm/kg H_2O

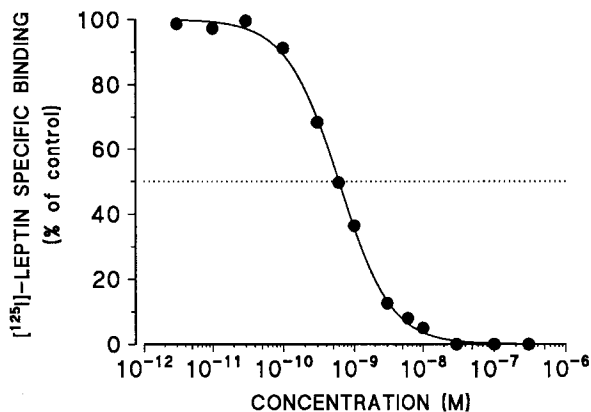


Fig. 3. Inhibition of specific [125 I]leptin binding to rat kidney medullary membranes by increasing concentrations of unlabeled leptin. Incubations were carried out with about 60 pM [125 I]leptin for 90 min at room temperature. Data are means calculated from a typical experiment performed in duplicate and repeated 5 times without noticeable changes.

($n = 15$) for the control and leptin-treated group, respectively. It is important to note that control parameter values observed in these studies are comparable to those previously described by ourselves and others [24,25].

4. Discussion

The present study demonstrates that the mature rat kidney expresses high-affinity binding sites for [125 I]leptin with intense localization in the medullary region. High levels of expression

occurred only in advanced postnatal developing stages and in the adult kidney. In addition, our data show that this hormone may play a direct role on this organ by inducing a significant diuretic effect after administration *in vivo*.

Widespread distribution of mRNA of several leptin receptor isoforms has been reported in several peripheral organs from mice, rat and human showing that this new hormone, besides having central effects, could have a pleiotropic action [26]. But up to now, only the leptin receptors of the choroid plexus and the hypothalamus have been further documented in terms of expression, binding affinity constants and mapping [5,11,27,28]. Here, we report the direct characterization and localization of leptin receptors in a target organ such as the kidney using [125 I]leptin as a ligand. Full identification of leptin receptors has been performed by *in vitro* binding of [125 I]leptin to rat renomedullary membranes and autoradiography on rat kidney sections demonstrating a preferential localization of leptin sites in the specific area of the inner medulla.

Binding of [125 I]leptin to rat renomedullary membranes showed high-affinity, specificity and saturability and was time-dependent. Dissociation of [125 I]leptin by unlabeled leptin occurred slowly and was not total after 4 h, suggesting a tight association of leptin to its sites. A single class of high-affinity binding sites with a K_d of 0.57 ± 0.14 nM and a maximal capacity (B_{max}) of 45 ± 10 fmol/mg protein ($n = 4$) was identified. Similar affinity was reported for murine choroid plexus leptin receptors transfected in COS cells (K_d value of 0.7 nM) [11] and in ob/ob mice choroid plexus (IC_{50} value of 1–2 nM) as determined by Lynn et al. [27]. In contrast, lower affinity was found for [125 I]leptin in purified hypothalamic ob/ob mice plasma membranes ($IC_{50} = 46$ nM) together with a

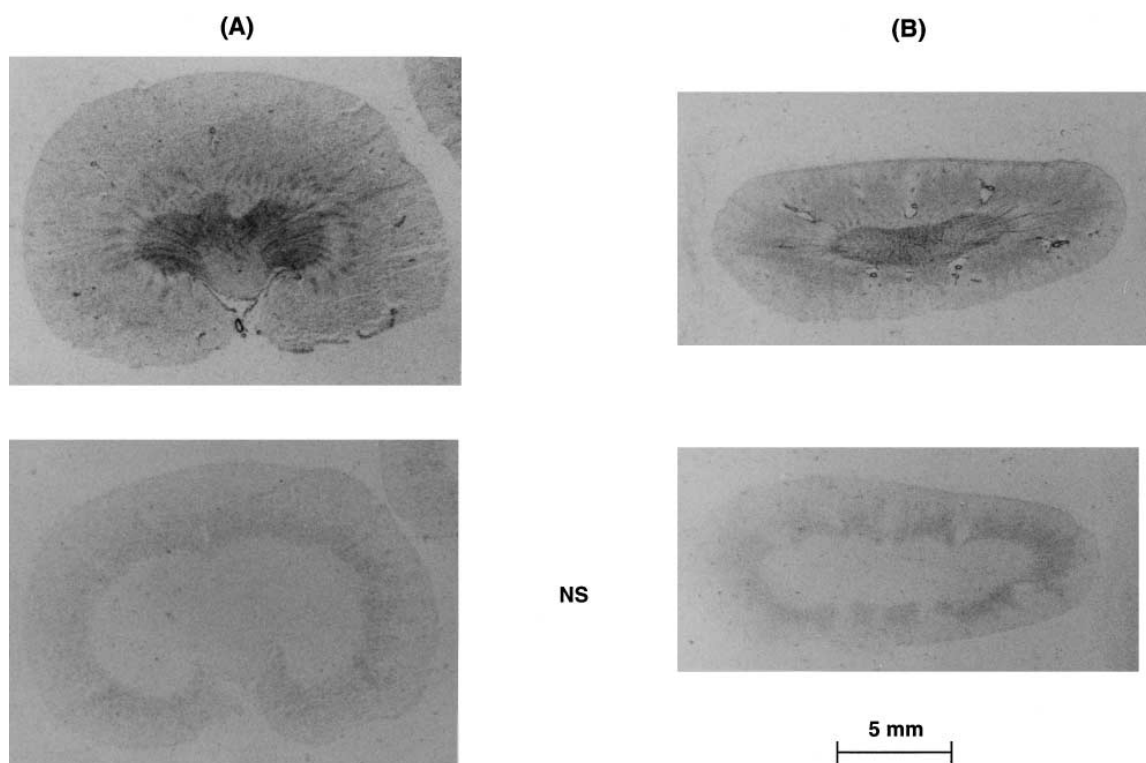


Fig. 4. Localization of [125 I]leptin binding sites in adult (60-day-old) rat kidney sections. Autoradiograms were obtained from (A) longitudinal or (B) transversal rat kidney sections incubated in the presence of 40 pM [125 I]leptin. Non-specific (NS) binding was obtained from the adjacent section by incubating in the presence of 0.3 μ M unlabeled leptin. Calibration bar: 5 mm.

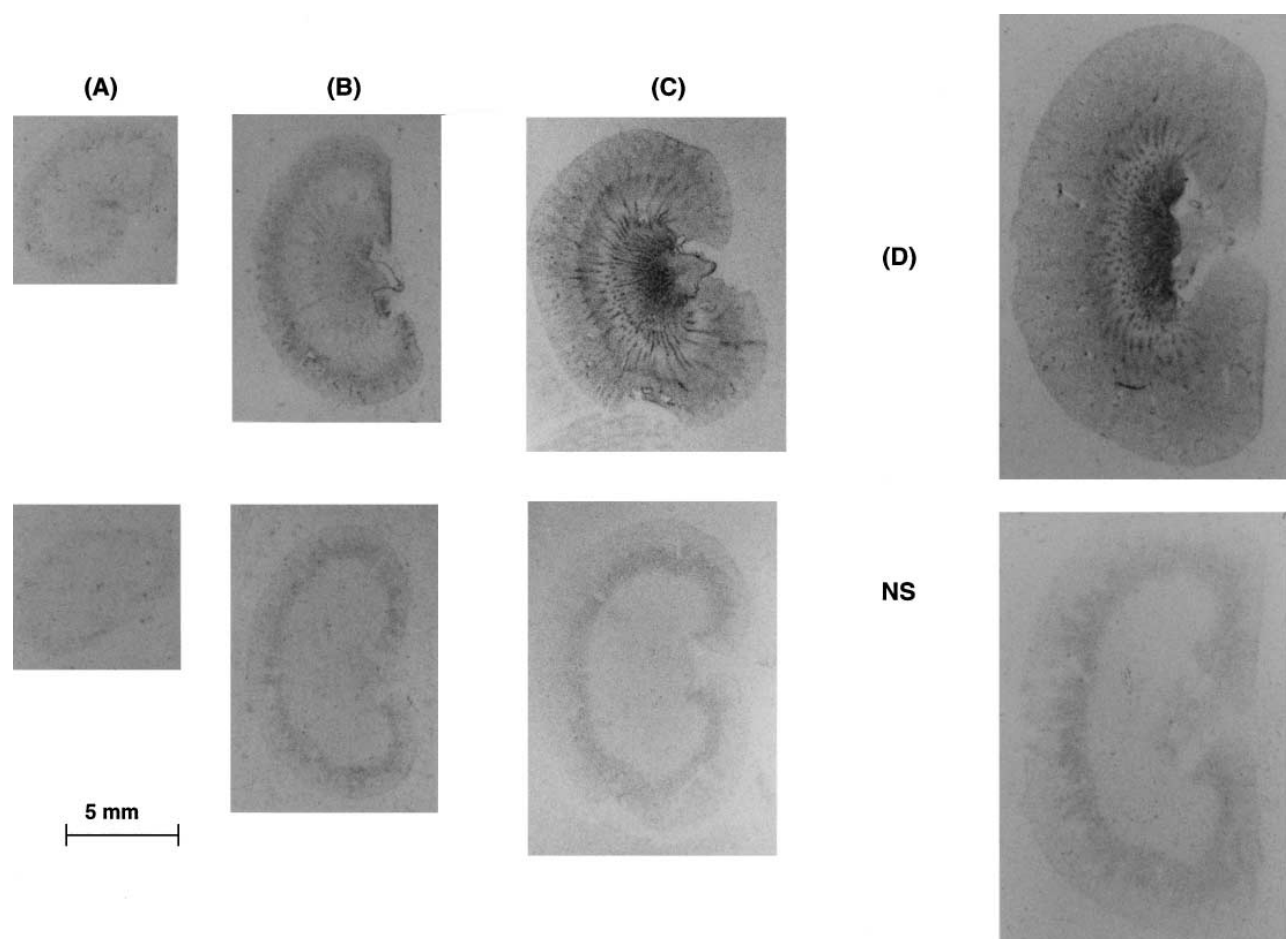


Fig. 5. Postnatal developmental expression of [125 I]leptin binding sites in the rat kidney. Autoradiograms were obtained from longitudinal kidney sections from 8-day (A), 21-day (B), 30-day (C) and 84-day-old (D) male Sprague-Dawley rats as described in Section 2. Each non-specific (NS) binding was obtained by incubating the adjacent section in the presence of 0.3 μ M unlabeled leptin. Calibration bar: 5 mm.

200-fold higher density of binding sites than that observed in our crude rat kidney preparations [5]. mRNA of a number of leptin receptor isoforms, with a different pattern of tissue expression, have been identified and seem highly conserved among species, including man [29]. A long isoform (ob-Rb, reported as the functional receptor) is preferentially expressed in the hypothalamus whereas short isoforms ob-Ra, ob-Rc, ob-Rd, ob-Re and rat-ob-Rf have selective expression in a wide variety of peripheral organs including the kidney [10–14,30]. Thus, it is tempting to speculate that the different leptin receptor isoforms could have different affinities for the natural hormone yielding different affinity for leptin in hypothalamus and in choroid plexus (or kidney). However, this explanation must be considered premature since so far only few binding data are available and different operating conditions could influence binding parameter determination. In ad-

dition, a recent study using [35 S]leptin as a probe reported equally potent binding in the choroid plexus from wild-type or leptin receptor-defective animals (db/db mice or Zucker rats) showing that the lack of response to leptin is not due to a deficiency in leptin binding but to an abnormality rather occurring at a site downstream from receptor binding [9].

Concerning detailed anatomic localization of leptin receptors in mature rat kidney, we observed that the inner medullary region bears the most abundant labeling, using macroscopic autoradiography. Although this technique did not allow localization at the cellular level, the distribution profile is consistent with labeling associated to vascular bundles, vasa recta and/or functional elements of the nephron such as the thin descending Henle's limb belonging to short and long-looped nephrons specific to the rat kidney anatomy. We cannot exclude the possibility of labeling in renomedullary inter-

Table 1
Effect of acute administration of leptin (1 mg/kg i.p.) on urine excretion in adult water-loaded Sprague-Dawley rats

Time (h)	Cumulated urine volume (ml)				
	0.5	1	2	4	6
Control	0.91 $\pm$ 0.33	3.05 $\pm$ 0.31	3.62 $\pm$ 0.45	3.86 $\pm$ 0.39	4.5 $\pm$ 0.48
Leptin	1.44 $\pm$ 0.30	3.14 $\pm$ 0.26	3.52 $\pm$ 0.22	3.16 $\pm$ 0.33	5.00 $\pm$ 0.35

Each group of rats were treated either with leptin or vehicle (bidistilled water, 1 ml/kg) just after receiving by gavage a water load of 25 mg/kg. Cumulated urine volume was measured at the different times. Result are means $\pm$ SEM of seven determinations.

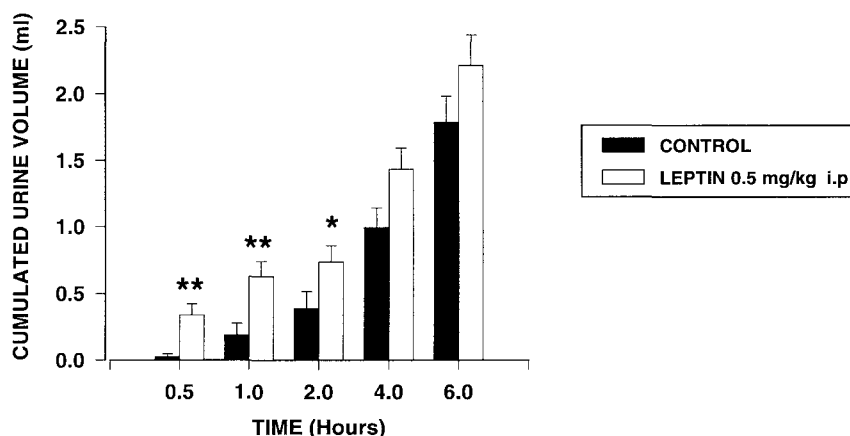


Fig. 6. Time-course effect of leptin on urine volume in normally hydrated rats. Each group of male Sprague-Dawley rats was treated either with leptin (0.5 mg/kg i.p.) or vehicle (bidistilled water, 1 ml/kg). Urine was collected throughout the 6-h period after treatment with individual measures of urine volume at 0.5, 1, 2, 4 and 6 h. Values are means $\pm$ SEM of 19 determinations per group. Drug and control groups were compared by a Mann-Whitney test, corrected by Bonferroni for repeated measures: * $P < 0.05$, ** $P < 0.01$.

stitial cells of the inner medulla. No leptin sites are evidenced, or are too scarce to be detected, in glomeruli, proximal and distal tubules and terminal collecting ducts. It is worth noting that vascular elements of the kidney also are a target system for interacting with leptin since the walls of big vessels of the cortico-medullary area are intensely labeled by [125 I]leptin. Even if up to now no major effect of leptin has been reported on arterial blood pressure (up to 1 mg/kg after i.v. administration in anaesthetized rats, not shown), specific association of [125 I]leptin to vascular structures has been reported in brain pia mater vessels from lean and obese Zucker rats [28].

After birth, we observed a gradual expression of renal leptin receptors as a function of maturation of this organ. Absent at early postnatal ages (8–21 days), full expression occurred after about 30 days and remained constant throughout adulthood, showing that an unknown induction mechanism regulates the plasticity of renal leptin receptors (hormones, growth factors, circulating leptin, etc.). Since several leptin receptor isoform mRNA coexist in the kidney (in particular, the mRNA of the two short isoforms ob-Ra ob-Rf are relatively abundant) [10–14,30], the individual postnatal developmental expression of each of them needs to be further explored by molecular biology. Up to now, no studies providing a detailed localization of the different mRNA isoforms are available and precludes any comparison between messenger distribution and receptor expression in the kidney. A comparative study in mice and man has shown that expression levels of the new isoform B219/ob-R in adult tissues are species specific and have lower expression in the whole adult human kidney than at fetal age. Obviously, differences in the pattern of tissue expression of receptor isoforms may reflect differences in their function.

Consistent with the presence of leptin receptors in the renomedullary region, we observed a significant diuretic effect lasting about 2 h at 0.5 mg/kg i.p., associated with a marked decrease in urine osmolality after *in vivo* leptin administration to mature normally hydrated conscious rats. Thus, we showed here that leptin, mainly known as a satiety factor, could have a functional significance in the kidney *in vivo* after systemic administration. Our results are in agreement with a recent abstract published during the preparation of this manuscript reporting a diuretic effect of leptin after direct infusion into the renal artery in anesthetized rats [31]. We can assume that

leptin could act directly via leptin receptors present in the area of renal medulla but a role in concert with other hormones cannot be excluded.

Thus, the functional role of leptin now appears to extend beyond the regulation of feeding behavior and metabolism to include other organs and biological functions (hematopoiesis, fertility, circadian rhythms, etc.). Two recent major observations show that leptin exerts a powerful stimulatory effect on the reproductive system and reverses the effects of fasting on other endocrine systems [12,32–34]. These findings are of interest since leptin plasma levels are submitted to fluctuation according to gender, age, circadian rhythm, and are markedly increased in various disorders such as obesity and in hemodialysis patients [7,35–38]. Moreover, it has been recently demonstrated that in lean subjects the circulating leptin is in protein bound form (among them the ob-Re soluble receptor isoform) subsequently not available for receptors, whereas in obese subjects, this hormone circulates mainly in a free form, corresponding presumably to the bioactive protein [39]. In this latter situation, we can assume that leptin could easily bind to target peripheral sites (i.e. in the kidney) and induce a potential biological effect or simply could be uptaken for clearance from the circulation.

In conclusion, we report here the presence and the characterization of high-affinity binding sites for leptin in the mature rat kidney, especially localized in the renomedullary region. The pattern of developmental plasticity showed that at adult and postnatal pre-adult ages high amounts of leptin receptors are expressed in this organ and could mediate the diuretic effect observed for leptin. However, the relationship between the appearance of renal leptin receptors and which isoforms, their linkage to the second messenger system, their function, circulating leptin levels and diuretic effect need to be further explored.

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