Characterization and quantitative topographical distribution of salmon calcitonin-binding sites in rat kidney sections

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Renal binding sites for labelled salmon calcitonin (sCT) were studied using cryostat sections and autoradiography. Increasing concentrations of unlabelled sCT inhibited ^{125}I-sCT binding. ^{125}I-sCT bound to a single site with a K_d of 2 nM and a number of sites of 220 fmol/mg protein. Mammalian calcitonins had low affinities and peptides unrelated to CT were devoid of any significant affinity for ^{125}I-sCT receptors. Autoradiograms disclosed a high concentration of ^{125}I-sCT receptors mainly located in the outer medulla and heterogeneously in the renal cortex. The distribution of specific binding sites is in agreement with the current concepts of renal action of calcitonin.

Calcitonin localization *Autoradiography* *Binding site* *(Kidney section, Rat)*

1. INTRODUCTION

Calcitonin [1], one of the 3 major hormonal regulators of calcium metabolism, interacts with specific receptors in kidney and bone [2–5]. Localization of these binding sites has relied upon the activation of adenylate cyclase in isolated mammalian nephron [6,7] or on autoradiographic studies following in vivo administration of labelled salmon calcitonin [8]. However, these approaches did not allow easy quantitation of the binding sites.

We have carried out quantitative in vitro autoradiography [9,10] on rat kidney cryostat sections to localize and quantitate calcitonin-binding sites.

Abbreviations: pCT, synthetic pig CT; hCT, synthetic human CT; VIP, vasoactive intestinal peptide; 1–34 PTH, parathyrine; TRH, thyrotropin-releasing hormone; CCK, cholecystokinin-8; Met-Enk, met-enkephalin; NT, neurotensin

2. MATERIALS AND METHODS

2.1. *Hormones and chemicals*

Synthetic salmon calcitonin (sCT, biological activity 4000 U/mg; batch no. 20051) was kindly supplied by Sandoz, Basle (Switzerland) and iodinated by the chloramine-T method [11] to a specific activity of 400 Ci/g. Na^{125}I (17 Ci/ng; 1 Ci = 37 × 10^{10} Bq) was obtained from New England Nuclear. The following reagents were used: denatured human serum albumin (Centre National de Transfusion Sanguine, Paris) and the selective enzymatic inhibitor, Antagosan (aprotinin, 2500 units antiplasmin corresponding to 100000 KIU/10 ml (Hoechst, France)). The following peptide hormones were used: purified porcine VIP (V. Mutt, Stockholm), 1–34 PTH synthetic (Touzart & Matignon, Paris), TRH (CEA, Saclay, France), Met-Enk (Peninsula, Belmont, CA), neurotensin 1–13 (J. Van Rietschoten, Marseille), hCT (Ciba, Switzerland), pCT (Armoor, USA).

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2.2. *In vitro incubation and autoradiography*

Male Wistar CF rats weighing 200–250 g were killed and kidneys removed, frozen and 20 μm sections were cut on a cryostat at −18°C, mounted on gelatin-coated slides, stored overnight at −20°C and then kept at −80°C until assayed. The slides were warmed at room temperature and incubated in 50 mM Tris-HCl buffer (pH 7.6) containing 1% denatured human albumin and 10% of Antagosan in the presence of 0.07 nm ^{125}I-sCT. Three sets of incubations were carried out. First, kidney sections were incubated with ^{125}I-sCT for various times, from 10 to 120 min to determine the optimum incubation time in the presence or absence of 10^{-7} M unlabelled salmon calcitonin. Second, sections were incubated with ^{125}I-sCT together with increasing concentrations of unlabelled sCT ranging from 10^{-10} to 10^{-6} M. Third, the radiolabelled peptide was incubated in the presence of various peptides ranging from 2×10^{-10} to 2×10^{-7} M to check the binding specificity and cross-reactivity. After incubation, the sections were washed 4 times with 40 mM Tris-HCl, pH 7.6, at 4°C for 2 min each. This washing procedure was found to give the best ratio between total and non-specific binding. The sections were removed from the slides with Whatman GF/B filter paper and placed in plastic tubes for counting (counting efficiency 50%).

2.3. *Quantitative autoradiography*

Sections treated as previously described were then dipped in distilled water and dried with cold air. Autoradiographs were prepared by a modification of the method of Rostène and Mourre [12] which facilitated quantitative absorbance measurements. The sections were placed in contact with [^{3}H]Ultrofilm (LKB) in Kodax X-ray film holders for 12 days at room temperature. The film was then developed and the density of the enlarged autoradiographic image measured using a densitometer which converts the amount of light into millivolts. The amount of ^{125}I-sCT-binding sites expressed as fmol/mg protein was measured by means of ^{125}I standards [12]. Proteins were measured by the method of Bradford [13]. Non-specific binding was calculated from the amount of ^{125}I-sCT bound in the presence of excess (10^{-7} M) unlabelled sCT. Results are expressed as means ± SE.

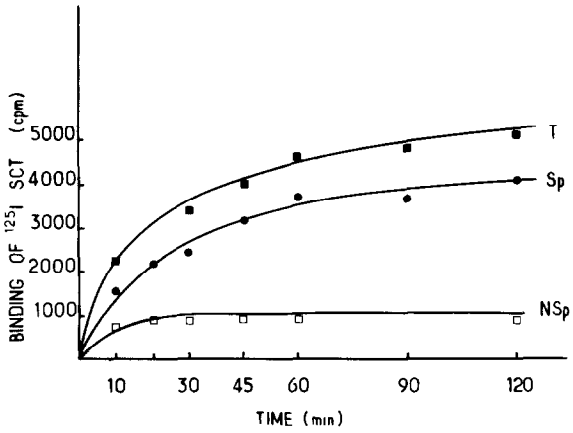

Fig.1. Binding of monoiodo salmon calcitonin (^{125}I-sCT) to rat kidney sections. Sections were incubated with 0.07 nm ^{125}I-sCT alone (T) or in the presence of 10^{-7} M unlabelled sCT (NSp). Specific binding (Sp) was determined as the difference between T and NSp. Each point is the mean of 3 determinations.

3. RESULTS

3.1. *In vitro binding of ^{125}I-sCT on sections*

3.1.1. Binding optimization

Incubation of ^{125}I-sCT with rat kidney sections reached equilibrium after 60 min at room temperature (fig.1). A routine incubation time of 75 min was then adopted for all subsequent experiments. Increasing concentrations of unlabelled sCT resulted in a dose-dependent displacement of ^{125}I-sCT from the sections (fig.2). Maximum displacement was obtained with 10^{-7} M sCT, equivalent to 80–85% of the total ^{125}I-sCT binding. Scatchard analysis [14] showed that under our experimental conditions, ^{125}I-sCT bound to a single class of binding sites with a dissociation constant of $2.1\pm0.2\times10^{-9}$ M and a number of binding sites of 218± 22 fmol/mg protein for 3 different experiments.

Two analogues of sCT, pig and human CT, were weak competitors for the ^{125}I-sCT-binding sites (fig.2). Other peptides which are unrelated to sCT showed no ability to displace ^{125}I-sCT (fig.2).

3.1.2. Autoradiographic studies

^{125}I-sCT-binding sites were mainly concentrated in the outer medulla. High densities of ^{125}I-sCT-

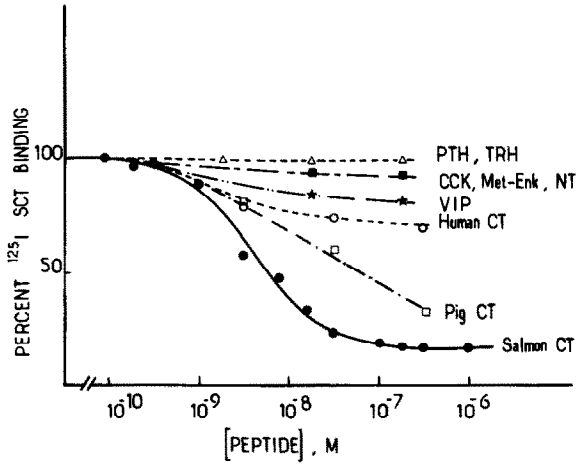

Fig.2. Displacement of ^{125}I-sCT by unlabelled peptides on rat kidney sections. Results were expressed as the percentage of the initial binding. Each point is the mean ± SE of 3 determinations.

binding sites were observed in the inner zone particularly rich in thick ascending limb segments and thin descending limb of the loop of Henle, as well as in the outer zone containing the thick ascending limb and the pars recta. Moderate amounts of binding sites were obtained in the medial part of the outer medulla. Fairly intense labelling of binding sites was also observed in the cortex but the label distribution in this part of the kidney was somewhat patchy and mainly concentrated in the superficial zone (fig.3A, table 1).

In the presence of an excess of unlabelled sCT,

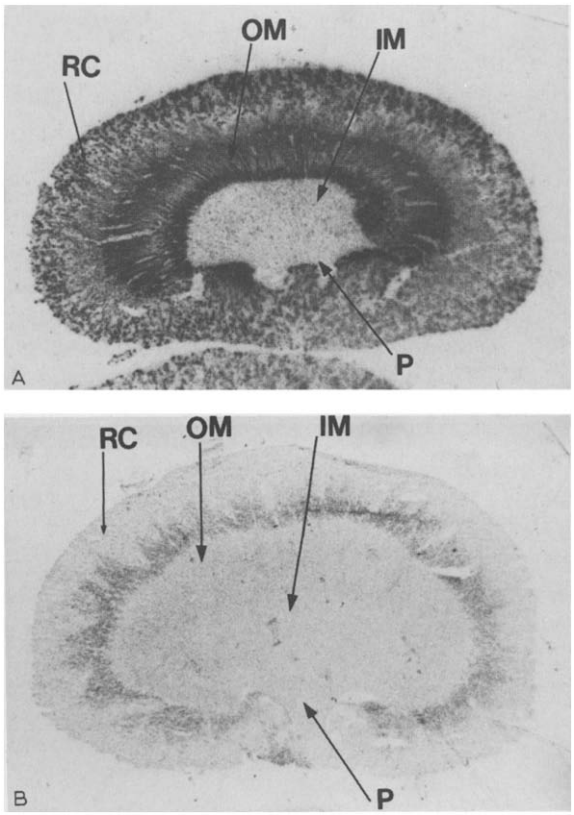

Fig.3. Autoradiograms of rat kidney sections (20 μm). RC, renal cortex; OM, outer medulla; IM, inner medulla; P, papilla. (A) Kidney sections incubated with 0.07 nM ^{125}I-sCT alone; (B) kidney sections incubated with 0.07 nM ^{125}I-sCT in the presence of 10^{-7} M unlabelled sCT. Binding conditions and procedures were as described in the text. Densitometric values obtained from the autoradiograms are reported in table 1.

Table 1

Localization and concentration of ^{125}I-sCT-binding sites in rat kidney sections

	Structures	^{125}I-sCT-binding sites (fmol/mg protein)
Cortex	PCT, DCT, CCT (superficial cortex)	17.26 ± 3.26
	cortical (TAL)	8.52 ± 0.98
Outer medulla	outer zone (TAL, pars recta)	34.07 ± 1.88
	middle zone (pars recta, TDL, TAL)	17.25 ± 1.43
	inner zone (TDL, TAL)	44.60 ± 2.00
Inner medulla	thin ascending limb	2.49 ± 0.21
Papilla	–	N.D.

Concentrations expressed as specific binding were measured by quantitative autoradiography (for details see section 2). PCT, proximal convoluted tubule; DCT, distal convoluted tubule; CCT, cortical collecting tubule; TAL, thick ascending limb; TDL, thin descending limb; N.D., non-detectable

the labelling almost disappeared from all levels with the exception of the juxtaglomerular region particularly rich in convoluted proximal tubules (fig.3B).

4. DISCUSSION

Previous experiments show that ^{125}I-sCT retains full biological activity [2–5] and thus can be used for identifying physiologically relevant renal receptor sites in rat kidney. The in situ labelling with ^{125}I-sCT is highly specific since incubation in the presence of an excess of unlabelled ligand results in a displacement of 85% of the labelled ligand, while no change is observed in the presence of peptides such as PTH, TRH, CCK, VIP, Met-Enk and NT. The results are in agreement with the specificity of sCT-binding sites previously observed with membrane preparations from rat kidney [2]. Furthermore, the displacement of labelled salmon calcitonin by unlabelled salmon calcitonin was much higher than that observed with mammalian calcitonin, such as pig or human, a factor which is consistent with the higher biological potency of the fish hormone as compared to mammalian hormones [2]. Under our conditions, pig CT seems to be more potent than human CT to displace ^{125}I-sCT binding. The topographical distribution of calcitonin-binding sites in the rat kidney as reported here is in agreement with previous observations based on calcitonin-sensitive adenylate cyclase activity and ion transport in the different parts of the renal tubule. The homogeneous labelling in the outer medulla is likely to correspond to binding to the thick ascending limb of the loop of Henle, where calcitonin stimulates adenylate cyclase activity, both in the rat and the rabbit [6,7]. In the cortex, the heterogeneous labelling represents binding to the cortical part of the loop of Henle, probably to cells of the distal and collecting cortical tubules. Adenylate cyclase activity is stimulated by calcitonin in all these tubular segments in the rat [15]. The non-specific labelling noted in the juxtaglomerular region which is particularly rich in convoluted proximal tubules, corresponds to an area where calcitonin has no physiological action [7].

We have thus demonstrated that ^{125}I-sCT may be used for studies on the nature and distribution of calcitonin-binding sites in rat kidney sections. While this initial study was performed on normal kidney tissue, there is every indication that we can adopt the same approach for the study of sCT-binding sites in a wide variety of physiological and pathological conditions related to changes in plasma CT concentrations.

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