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Identification and Characterization of Opioid and Somatostatin Binding Sites in the Opossum Kidney (OK) Cell Line and Their Effect on Growth

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Abstract Opioids and somatostatin analogs have been implicated in the modulation of renal water handling, but whether their action is accomplished through central and/or peripheral mechanisms remains controversial. In different cell systems, on the other hand, opioids and somatostatin inhibit cell proliferation. In the present study, we have used an established cell line, derived from opossum kidney (OK) proximal tubules, in order to characterize opioid and somatostatin receptors and to investigate the action of opioids and somatostatin on tubular epithelial tissue. Our results show the presence of one class of opioid binding sites with kappa$_1$ selectivity (K_D 4.6 ± 0.9 nM, 57,250 sites/cell), whereas delta, mu, or other subtypes of the kappa site were absent. Somatostatin presents also a high affinity site on these cells (K_D 24.5 nM, 330,000 sites/cell). No effect of either opioids or somatostatin on the activity of the Na^+/P_i cotransporter was observed, indicating that these agents do not affect ion transport mechanisms. However, opioid agonists and somatostatin analogs decrease OK cell proliferation in a dose-dependent manner; in the same nanomolar concentration range, they displayed reversible specific binding for these agents. The addition of diprenorphine, a general opioid antagonist, reversed the effects of opioids, with the exception of morphine. Furthermore, morphine interacts with the somatostatin receptor in this cell line too, as was the case in the breast cancer T47D cell line. Our results indicate that in the proximal tubule opioids and somatostatin do not affect ion transport, but they might have a role in the modulation of renal cell proliferation either during ontogenesis or in kidney repair.

Key words: opossum kidney cells, cell proliferation, opioids, opioid receptors (delta, mu, kappa), somatostatin, somatostatin receptors, cell proliferation

The majority view implicates central somatostatin and opioid actions to be responsible for alterations in kidney function. It remains, however, possible that these mediators exert direct effects on renal cells. Indeed, opioid and somatostatin binding sites have been detected in the kidney, on renal blood vessels, and on the sympathetic nerves innervating the organ [Kapusta et al., 1989; Kapusta and Obih, 1993, 1995a,b]. Somatostatin and opioids have been reported to modulate water excretion (Ashton et al., 1989; Bruno et al., 1993; Di Bona and Jones, 1994; Hayes et al., 1987; Quirion et al., 1983; Reubi et al., 1993; Salas et al., 1989; Slizgi and Ludens, 1985; Tulassay et al., 1991; Vora et al., 1986; Yamada et al., 1989] but not to affect renal sodium or potassium handling.

The opossum kidney cell line (OK cells) is a well-characterized renal tubular cell line retaining several characteristics of the normal proximal tubules. We have recently shown that in these cells activation of Na^+/P_i cotransport induces acute and short-lived cytoskeletal protein modifications [Papakostanti et al., 1996]. It is therefore a good model to investigate the presence and potential functions of neuropeptide receptors in renal epithelial cells.

In the present work, we report the existence of kappa-opioid and somatostatin binding sites on growing OK cells. We further observed that both opioids and somatostatin decrease cell

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growth but do not affect Na^+/P_i cotransport. Finally, we demonstrated that some opioids (morphine) interact with the somatostatin receptor system in this cell line too, as was previously shown for breast cancer cells [Hatzoglou et al., 1995].

MATERIALS AND METHODS

Cell Cultures

Opossum kidney (OK) cells were from American Type Culture Collection (Rockville, MD) and were studied between passages 40 and 50. The cells were maintained in a humidified atmosphere of 5% CO_2–95% air at 37°C and fed twice weekly with a 1:1 DMEM:HAM's F12 medium, which contained 1 mM P_i, supplemented with 10% v/v fetal calf serum and 2 mM glutamine, 20 mM $NaHCO_3$, 22 mM HEPES, 50 IU/ml penicillin, and 50 mg/ml streptomycine. Cells from high density cultures were seeded into six-well culture dishes, unless otherwise stated, for proliferation and binding experiments. Culture media were changed every 48 h prior to the administration of drugs and daily during the proliferation assays.

Binding Assays

Opioid binding. Opioid binding was performed on whole cells as described previously [Hatzoglou et al., 1995]. Briefly, cells were seeded in six-well dishes at a density of 10^6 cells/well. Binding was performed in phosphate-buffered saline (10 mM phosphate, 150 mM NaCl, pH 7.4) in a total volume of 0.4 ml containing radioactive opioid without or with a thousandfold molar excess of the same nonlabeled opioid and effectors (see the following section). For saturation binding, at least eight to ten points with different concentrations of radiolabeled opioid (varying from 1–50 nM) were performed in duplicate. The cells were incubated for 2 h at room temperature (18–20°C). At the end of the incubation period, the unbound radioactivity was eliminated by washing the cells twice with 2 ml cold phosphate buffer. Cells were removed from plates with 0.5 ml 2 N NaOH and mixed with 4 ml scintillation cocktail (Sigma Fluor; Sigma Chemical Co., St. Louis, MO). The bound radioactivity was counted in a scintillation counter (Tricarb Series 4000; Packard, Downers Grove, IL) with a 60% efficiency for [^{3}H]. Binding measurements were repeated at least twice (in duplicate), and the results were analyzed by the Origin V 3.78 package (MicroCal Co., MA) using equations described by Munson and Rodbard [1980].

Specific conditions for differential detection of various types of opioid binding sites. The conditions used for the differential detection of various opioid sites were described in previous studies from our group [Hatzoglou et al., 1996]. Briefly, delta opioid sites were detected by the use of tritiated [D-Pen^2, D-Pen^5]enkephalin (DPDPE). Mu sites were detected by the selective ligand [D-Ala^2, N-Me-Phe^4, Gly^5-ol]enkephalin (DAGO). The interaction of tritiated ethylketocyclazocine with the $kappa_1$ site was investigated by performing the binding in the presence of 10 μM [D-Ala^2, D-Leu^5]enkephalin (DADLE). Under these conditions, we and others have reported that DADLE masks delta, mu, and $kappa_2$ sites on which ethylketocyclazocine could bind with high affinity. Finally, [^{3}H]diprenorphine binding (reacting mainly with delta, mu $kappa_2$, and $kappa_3$ sites) was determined under the same conditions as for ethylketocyclazocine. The interaction of the ligand with the $kappa_3$ site was estimated in the presence of 10 μM DADLE.

Somatostatin binding conditions. One million cells/well in monolayer were used for saturation and displacement binding experiments, as reported previously [Hatzoglou et al., 1995]. Before binding, cells were washed twice with 2 ml of phosphate-buffered saline. Binding was performed in phosphate-buffered saline, as for opioids, in a total volume of 0.5 ml, containing [^{125}I]Tyr^{11} somatostatin-14, without (total binding) or with a thousandfold molar excess of somatostatin-14 (nonspecific binding). For saturation binding, at least eight to ten points with different concentrations of radiolabeled peptide were performed in duplicate. The cells were incubated for 2 h at room temperature (18–22°C). At the end of the incubation period, the unbound radioactivity was eliminated by washing the cells twice with 2 ml cold phosphate buffer. Cells were removed from plates with 0.4 ml 2 N NaOH, and the bound radioactivity was counted in a gamma counter (Tricarb Series; Packard), with a 95% efficiency for ^{125}Iodine. Binding was repeated at least three times, and the results were analyzed by the Origin V 3.78 package (MicroCal Co.) using equations described by Munson and Rodbard [1980].

Measurement of Cell Growth

Cells were plated in 24-well plates at an initial density of 25×10^3 cells/well supplemented with 1 ml medium/well. All drugs were added to

cultures 1 day after seeding (designated as day 0) to ensure uniform attachment of cells at the onset of the experiments. Cells were grown for a total of 4 days, with daily change of the medium containing opioid drugs or somatostatin analogs. All added drugs were dissolved shortly before use.

Cell growth was measured by the tetrazolium salt assay [Mosmann, 1973]. Cells were incubated for 4 h at 37°C with the tetrazolium salt (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide), and metabolically active cells reduced the dye to purple formazan. Dark blue crystals were dissolved with propanol. The absorbance was measured at 570 nm and compared against a standard curve of known numbers of T47D cells. All experiments were performed at least three times in triplicate.

P_i Uptake Experiments

Cells treated with ethylketocyclazsocine or sandostatin at concentrations ranging from 10^{-12} to 10^{-6} M for 4 days were washed twice with uptake solution (150 mM NaCl, 1.8 mM $MgSO_4$, 1 mM $CaCl_2$, and 10 mM HEPES, pH 7.4). For P_i uptake measurements, incubations were carried out for 5 min after the addition of radioactively labeled 0.1 mM $K_2H^{32}PO_4$. P_i uptake was stopped by rapidly washing the cells three times with 2 ml of ice-cold stop solution, the composition of which was identical to the uptake medium, with the exception of NaCl which was substituted equimolarly by choline chloride. Cells were solubilized with 0.4 ml of 0.5% Triton X-100, and the radioactivity was counted in 100 μl aliquots by liquid scintillation in a Tricarb Series 4000 counter (Packard) with a 60% efficiency for [^{3}H] [Papakostanti et al., 1996].

Radiochemicals and Chemicals

[^{3}H]ethylketocyclazocine (S.A. 18 Ci/mmol), [^{3}H][D-Pen^2, D-Pen^5]enkephalin (S.A. 37 Ci/mmol), and $K_2H[^{32}P]O_4$ (specific activity 1,000 Ci/mmol) were bought from New England Nuclear Co. (Boston, MA). [^{3}H]diprenorphine (S.A. 29 Ci/mmol), [D-Ala^2, N-Me-Phe^4, Gly^5-ol]enkephalin (S.A. 60 Ci/mmol), and [^{125}I]-Tyr^{11}-somatostatin-14 (2,000 Ci/mmol) were obtained from Amersham (Buckinghamshire, UK). Cell culture media were from Gibco (Grand Island, NY). The somatostatin peptides were obtained from Bissendorff Biochemicals (Hanover, Germany). Sandostatin was a gift from Sandoz (Basel, Switzerland). All other peptides were from Sigma Co. All chemicals were from Merck (Darmstad, Germany). Ethylketocyclazocine was a gift from Sterling-Winthrop (Rensselaer, NY). Diprenorphine and etorphine were from Reckit and Coleman Co. Morphine and naloxone were from Francopia.

RESULTS

Characterization of Opioid Binding Sites on OK Cells

As shown in previous studies from our group [Hatzoglou et al., 1996], the use of a number of opioid ligands with variable selectivities for opioid sites, applied in combination with rather selective effectors, is of great value in identifying opioid binding sites, both in membrane preparations and whole cells. We have used this same methodology to seek opioid binding sites in OK cells. Table I indicates that indeed we were able to identify and characterize opioid sites on these cells, and analysis of our findings show the following.

1. [^{3}H][D-Pen^2, D-Pen^5]enkephalin (DPDPE) (a ligand which in nanomolar concentrations binds to delta opioid sites), [^{3}H][D-Ala^2, N-Me-Phe^4, Gly^5-ol]enkephalin (DAGO) (a specific mu ligand), and [^{3}H]diprenorphine (an almost universal ligand of opioid sites presenting a selectivity for delta, mu kappa$_2$, and kappa$_3$ opioid sites) do not show any specific binding on OK cells (Table I).
2. [^{3}H]ethylketocyclazocine, a ligand of delta, mu, kappa$_1$, and kappa$_2$ opioid sites, demonstrates a monophasic binding curve (Fig. 1 top panel; Table I) with an affinity of 4.6 ± 0.9 nM and a binding capacity of 230 ± 22 pM, corresponding to 57,250 sites/cell. Ethylketocyclazocine binds to delta, mu, kappa$_1$, and kappa$_2$ opioid sites. The addition of micromolar concentrations of [D-Ala^2, D-Leu^5]enkephalin during the binding could mask all sites with the exception of kappa$_1$ [Castanas et al., 1985]. Therefore, when saturation binding is performed in the presence of [D-Ala^2, D-Leu^5]enkephalin (10 μM), the ligand interacts only with kappa$_1$ sites. The results of this binding assay are presented in Figure 1 (bottom panel) and in Table I. Under these conditions, no significant variation of binding parameters was observed. On the contrary, the addition of 5 μM U69593,

TABLE I. Characterization of Opioid Binding Sites on OK Cells (Mean ± SE of Three Experiments in Duplicate)

Ligand	Effector	K_D (mM)	Bmax (pM)	Sites
[^{3}H]ethylketocyclazocine	—	4.6 ± 0.9	230 ± 22	δ, μ, κ1, κ2
	DADLE (10 μM)	3.5 ± 0.5	289 ± 34	κ1
	U69593	ND	ND	κ1
[^{3}H]diprenorphine	—	ND	ND	δ, μ, , κ2, κ3
[^{3}H]DPDPE	—	ND	ND	δ
[^{3}H]-DAGO	—	ND	ND	μ

ND = Not Detected.

a selective kappa$_1$ agonist, during [^{3}H]ethylketocyclazocine binding abolishes all specific binding.

Displacement of radiolabeled ethylketocyclazocine by unlabeled ethylketocyclazocine and U69593, presented in Figure 2, confirmed the existence of one site. Indeed, these two substances displace [^{3}H]ethylketocyclazocine in a monotonous manner, with IC_{50}s of 2.4 and 1.69 nM, respectively. These results, taken together, indicate that only kappa$_1$ sites are present on membranes of OK cells.

Characterization of Somatostatin Binding Sites on OK Cells

Figure 3 presents the saturation binding isotherm of [^{125}I]Tyr^{11}-somatostatin-14 on whole OK cells. As shown (Fig. 3, top pannel), binding was monophasic. Analysis of binding in Scatchard coordinates (Fig. 3, top pannel, insert) revealed the existence of one population of binding sites with K_D of 24.5 nM and a binding capacity of 1.058 nM, or 330,000 sites/cell. Displacement of [^{125}I]Tyr^{11}-somatostatin-14 by somatostatin 28 and sandostatin (SMS 201-995) with IC_{50}s 1.25 and 3.8, respectively, confirmed the existence of a one site model (Fig. 3, bottom pannel).

Effects of Opioids and Somatostatin on Na^+/P_i Cotransporter

We have further investigated whether opiate and somatostatin agonists could modify the activity of the Na^+/P_i cotransporter. As indicated in Table II, in the presence of opioids or somatostatin no significant changes in [$^{32}P_i$] uptake by the cells were observed. Our findings indicate that these agents do not affect Na^+/P_i cotransport under the conditions of the present study.

Effect of Opioid and Somatostatin Agonists on Proliferation of OK Cells

Figure 4 presents the effect of opioid receptor agonists on proliferation of OK cells after 3 days. As shown, ethylketocyclazocine, at concentrations varying from 10^{-12} to 10^{-6} M, inhibits in a dose-dependent manner the proliferation of these cells. This effect is antagonized by the addition of 10^{-6} M diprenorphine, a general opioid receptor antagonist. Similar results were obtained by the specific kappa$_1$ opioid receptor agonist U69593. [D-Ser^2, Leu^5]enkephalin, Thr^6 (DSLET) (a delta agonist) produces minimal effects at much higher concentrations equally antagonized by diprenorphine. On the contrary, the addition of morphine produced a dose-dependent inhibition of cell proliferation, but its effects were not reversed by diprenorphine. Somatostatin-28 and sandostatin (SMS 201-995) also caused a dose-dependent inhibition of cell proliferation. This effect was not due to a cytotoxic action of these drugs on the OK cells, as shown in Figure 5. Indeed, opioids and sandostatin decrease the final cell number, while the slope of the obtained growth curves remains unchanged (Fig. 5, insert).

Interaction of Morphine With Somatostatin Binding Sites

In a previous work, we have shown that morphine, even in the absence of mu binding sites, can produce a decrease in cell proliferation by interacting with type II somatostatin receptors [Hatzoglou et al., 1995]. Here we report analogous dose-dependent effects of morphine on OK cell growth, which remained unaffected by opioid antagonists. We have therefore investigated potential morphine interactions with somatostatin receptors in OK cells analogous to those observed in the breast cancer T47D cell line. Our results, depicted in Figure 6, show that

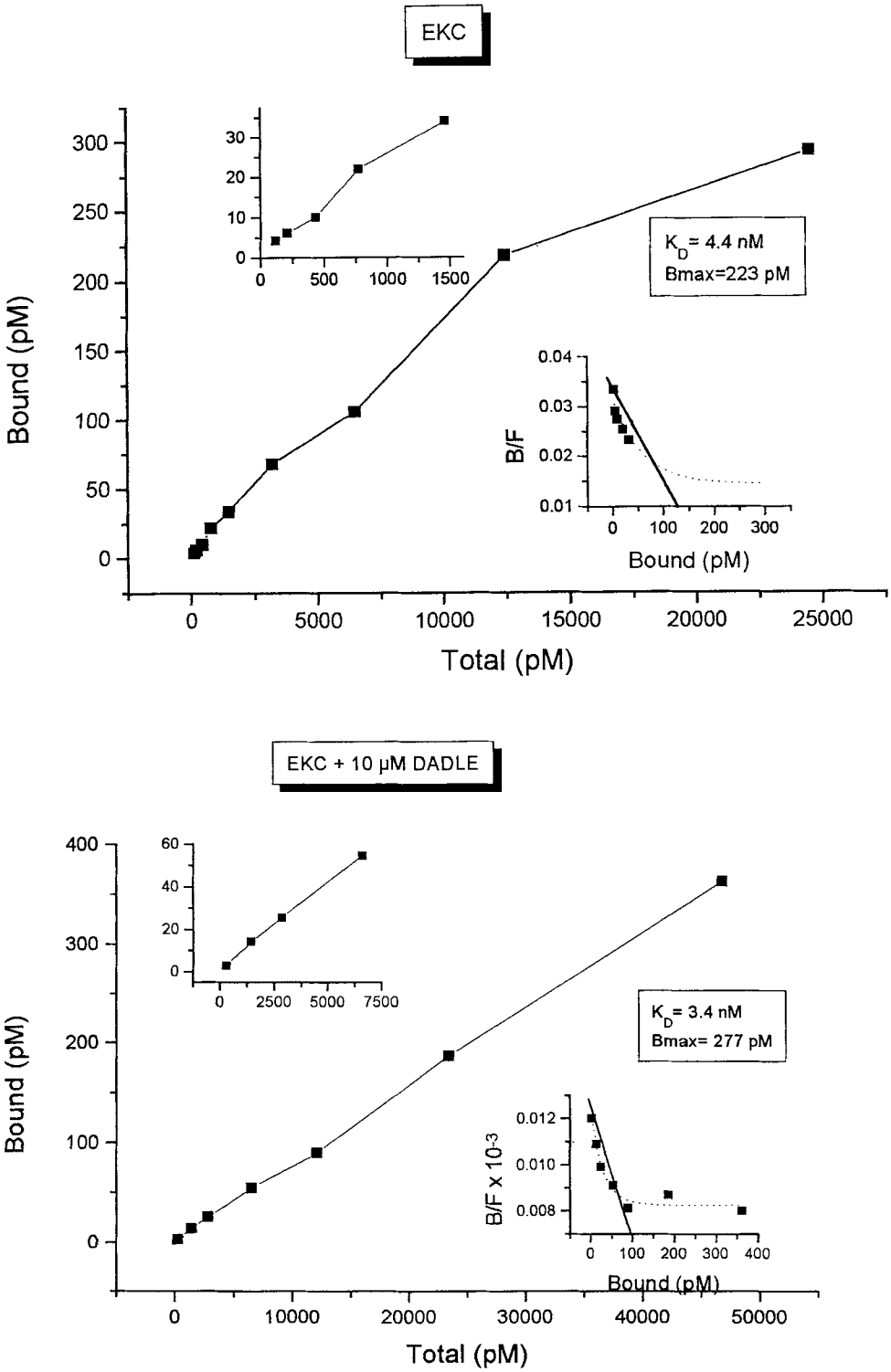

Fig. 1. Saturation binding of [^{3}H]ethylketocyclazocine to opossum kidney (OK) cells. One million cells were incubated for 2 h at room temperature with increasing concentrations of [^{3}H]ethylketocyclazocine (1–50 nM) in phosphate-buffered saline, pH 7.4. **Top panel:** Binding of ethylketocyclazocine in the absence of any effector. **Bottom panel:** Analogous experiments in the presence of 10 μM [D-Ala^2, D-Leu^5]enkephalin, which masks the binding to delta, mu, and kappa$_2$ opioid sites. In each panel, the **top insert** presents a magnification of the initial part of the saturation isotherm, while the **bottom insert** shows the analysis of data in Scatchard coordinates. See Materials and Methods and Results for further details. The figure presents results of a typical experiment in duplicate.

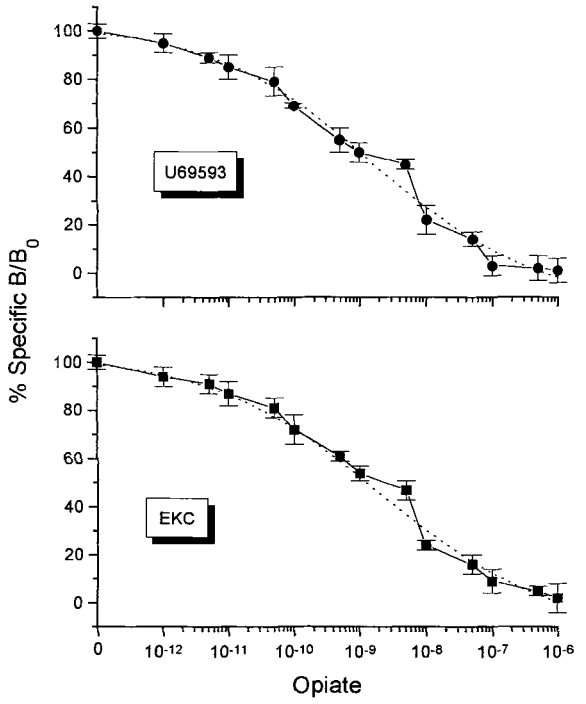

Fig. 2. Displacement of [^{3}H]ethylketocyclazocine by ethylketocyclazocine (EKC) and U69593. [^{3}H]ethylketocyclazocine (1.2 pmoles) (50,000 cpm) were incubated with OK cells in the presence of the indicated concentrations of U69593 or unlabeled ethylketocyclazocine. The figure presents the mean of two displacement experiments in triplicate. Bars show the standard error of the mean.

morphine indeed displaced [^{125}I]Tyr^{11}-somatostatin-14 in a dose-dependent manner and in parallel to its inhibition of cell proliferation (compare Figs. 4 and 6).

DISCUSSION

Previous studies have shown the existence of specific opioid binding in homogenates and slices of rat kidneys. Opioid sites characterized in these initial experiments bind with high affinity ethylketocyclazocine and other kappa opioids [Quirion et al., 1983; Slizgi and Ludens, 1985]. A close correlation was found between opioid binding and water excretion in the rat [Slizgi and Ludens, 1985]. Furthermore, water deprivation seems to increase the number of opioid (beta-endorphin) binding sites without change in their affinity [Dave et al., 1985]. Ribstein and Humphreys [1983] report that after controlateral nephrectomy in rats, modulation of sodium excretion involves opiate receptors. However, recent studies in humans have shown that this effect might be mediated indirectly, through alterations in the circulating levels of atrial natriuretic peptide [Grzeszczak et al., 1994]. The opioid antagonist naloxone impairs the adaptive response to sodium restriction [Di Bona and Jones, 1994]. However, central effects and mechanisms involving renal nerve traffic have also been recognized during maximal salt retention or natriuretic response consequent to saline volume expansion, respectively [Kapusta and Obih, 1993, 1995a,b]. Ashton et al. [1989], Salas et al. [1989], and Yamada et al. [1989] have reported that kappa opioids (bremazocine) produce water diuresis, without affecting total Na^+ and K^+ excretion, through both central and local mechanisms. By investigating the opioid-related effect under combinations of opioid agonists and antagonists, Hayes et al. [1987] have shown that renal mu- and kappa-type opioid receptors might be implicated in water excretion, while Kapusta et al. [1989] suggested that these effects are mediated by postganglionic renal nerves. The main central mechanism proposed for the effect of opioids on renal water handling is inhibition of vasopressin secretion, whereas a direct tubular local competition or interaction cannot be excluded. Furthermore, a peripheral interaction of endogenous opioids with angiotensin II receptors and a direct stimulatory effect on renal tubule carbohydrate metabolism were also reported [Hothi et al., 1989].

High affinity somatostatin receptors of types III and IV [Bruno et al., 1993] have also been found in human renal tubules and vasa recta [Reubi et al., 1993] and the rabbit renal papilla [Arilla et al., 1986]. They have been implicated in promoting water excretion [Tulassay et al., 1991; Vora et al., 1986], the modulation of vasopressin action [Brautbar et al., 1979; Reid and Rose, 1977], and the tonic inhibition of the renin-angiotensin system [Mazzocchi et al., 1992] as well as the reabsorption of sugar and amino acids by the renal tubules [Reubi et al., 1993]. Berthold and del-Poso [1989] reported a direct antidiuretic effect of somatostatin analogs in humans. High affinity somatostatin receptors have also been found in renal carcinomas [Reubi and Kvols, 1992] and in veins surrounding them [Reubi et al., 1994] and have been implicated in the modification of tumor-host interactions.

In addition to the existence of specific binding sites, opioid and somatostatin peptides themselves also have been detected in the kidney. Proenkephalin B–related mRNA was found in porcine kidney [Pittius et al., 1987], while immunoreactivities for beta-endorphin, dynorphin, and Met^5-enkephalin were detected in rat kid-

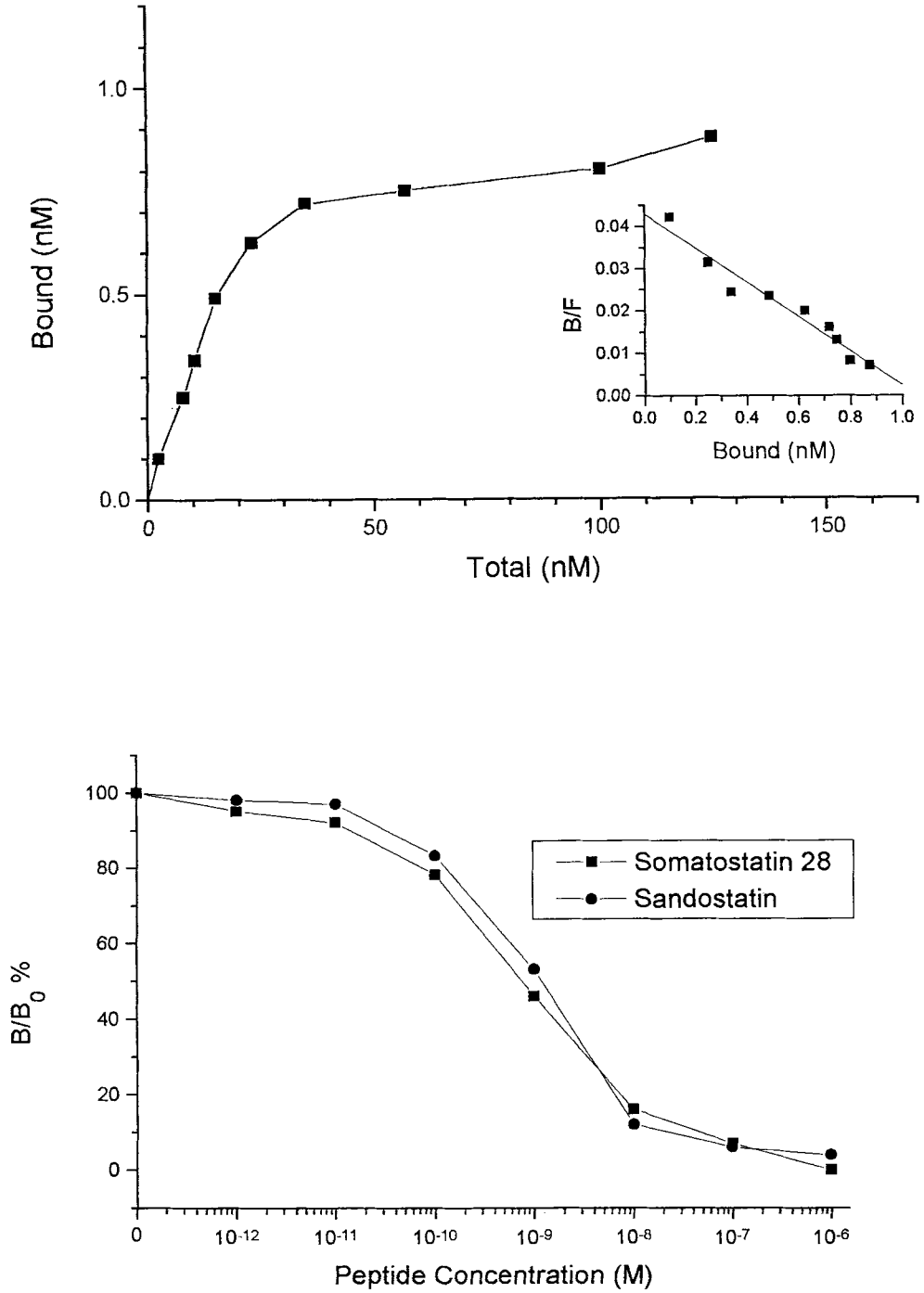

Fig. 3. Characterization of somatostatin binding sites on opossum kidney (OK) cells. One million cells were incubated with [^{125}I]Tyr^{11}-somatostatin-14 for 2 h at room temperature. **Top panel:** Saturation binding of [^{125}I]Tyr^{11}-somatostatin-14. **Insert:** Analysis of the presented binding isotherm in Scatchard coordinates. **Bottom panel:** Displacement of [^{125}I]Tyr^{11}-somatostatin-14 binding by somatostatin-28 and sandostatin (SMS 201-995). Results of a typical experiment. See Materials and Methods and Results for further details.

ney [Bhargava et al., 1988]. Interestingly, proenkephalin A is expressed transiently in different mesodermal lineages in mice, including that which develops to become the kidney. Both mRNA and Met^5-enkephalin can be detected, and it is proposed that opioids might play a role during renal development and organogenesis [Keshet et al., 1989; Kew and Kilpatrick, 1990]. Neutral endopeptidase (enkephalinase), which is also present in the kidney [Sales et al., 1991],

TABLE II. Effect of Opioid and Somatostatin Analogs on the Na^+/P_i Cotransporter*

Concentration (M)	Ethylketocyclazocine	Sandostatin
0	20.57 ± 1.2	20.18 ± 0.9
10^{-12}	19.85 ± 0.9	18.03 ± 0.5
10^{-11}	19.45 ± 0.9	17.89 ± 0.6
10^{-10}	18.67 ± 0.5	20.08 ± 1.0
10^{-9}	20.97 ± 0.9	16.98 ± 0.5
10^{-8}	20.99 ± 0.6	17.62 ± 0.3
10^{-7}	19.96 ± 0.5	20.71 ± 0.9

*The table presents the $^{32}P_i$ uptake in OK cells expressed in picomoles/milligram of protein after treatment with the indicated concentrations of ethylketocyclazocine and sandostatin for 4 days (mean ± SE of two experiments in duplicate). See Materials and Methods for experimental details.

in addition to the breakdown of endogenous opioids, is involved in the inactivation of other neuropeptides, including atrial natriuretic peptide, substance P, and somatostatin. Somatostatin immunoreactivity has been observed in single neural varicosities of renal hilus artery [Reinecke and Forssmann, 1988] but not in renal neurons [Chevendra and Weaver, 1992].

In the present study, we have used the opossum kidney cell line (OK cells) in order to characterize opioid and somatostatin receptors. Our results, in accord with studies mentioned above, show the existence of high affinity $kappa_1$ opioid receptors. We could not detect any delta or mu binding under the experimental conditions used. The affinity of kappa opioid binding (about 4 nM) is comparable with that reported by Quirion et al. [1983] (7.3 nM), but it differs from the two-site model for the binding of beta-endorphin in rat kidney (0.68 and 210 nM) presented by Sato et al. [1990]. This discrepancy might be attributed to the homogeneous population of cells used in the present work, while previous studies dealt with preparations from organs and crude homogenates that contained membranes from different cell types and also nerve terminals. One should additionally consider the possibility of species differences. Thus, Dissanayake et al. [1991] have reported species and receptor type differences between the guinea pig and the rat concerning kidney opioid binding. It is noteworthy, however, that we were able to confirm the results of Reubi et al. [1993] by identifying only one high affinity somatostatin binding site (Fig. 3).

In a number of species, both opioid and somatostatin receptors have been implicated in the modulation of water excretion. On the other hand, in different studies, no effect on Na^+ excretion was found [Ashton et al., 1989; Hayes et al., 1987; Salas et al., 1989; Slizgi and Ludens, 1985; Tulassay et al., 1991; Vora et al., 1986; Yamada et al., 1989]. As the OK cell line expresses the Na^+/P_i cotransporter [Papakostanti et al., 1996], we have investigated whether opioids or somatostatin agonists affect Na^+/P_i cotransport. Our results (Table II) show that neither somatostatin nor opioid agonists modify the cotransport of these ions through the cell membrane. These data are in accord with previous investigations [Salas et al., 1989; Tulassay et al., 1991; Vora et al., 1986] and indicate that, in our experimental model and under the conditions used, opioids and somatostatin do not affect ion transport.

Recently, we [Hatzoglou et al., 1995, 1996] and others [Maneckjee et al., 1990] have suggested that opioids and somatostatin can decrease breast tumor cell proliferation by interacting with specific binding sites. In addition, certain opioids were found to bind to the type II somatostatin receptor of such cells [Hatzoglou et al., 1995]. In the present study, we have further investigated the influence of opioids and somatostatin on the proliferation of OK cells. As shown in Figure 3, all agonists inhibit cell proliferation. The most active compounds, under our experimental conditions, were ethylketocyclazocine and U69593, followed by morphine, somatostatin-28, and sandostatin, while [D-Ser^2, Leu^5]-enkephalin, Thr^6 (DSLET) (a delta agonist) showed minimal effects. These results indicate that the opioid and somatostatin binding sites described on OK cells may indeed have biological significance, qualifying as true receptors.

Recent results have shown interactions of certain opioids (morphine) with somatostatin receptors [Hatzoglou et al., 1995]. In the present study, in spite of the absence of mu opioid receptors, morphine produced a dose-dependent inhibition of cell growth. We have looked therefore for a possible interaction of morphine with somatostatin receptors in OK cells, similar to the one observed in the T47D human breast cancer cell line. Our results confirm that, in OK cells too, morphine can displace somatostatin-14 from its binding sites. This observation suggests that the interaction of morphine with somatostatin receptors is not limited to a cancer cell line but might be a general characteristic of a whole family of homologous receptors. Interestingly, somatostatin and opioid receptors present con-

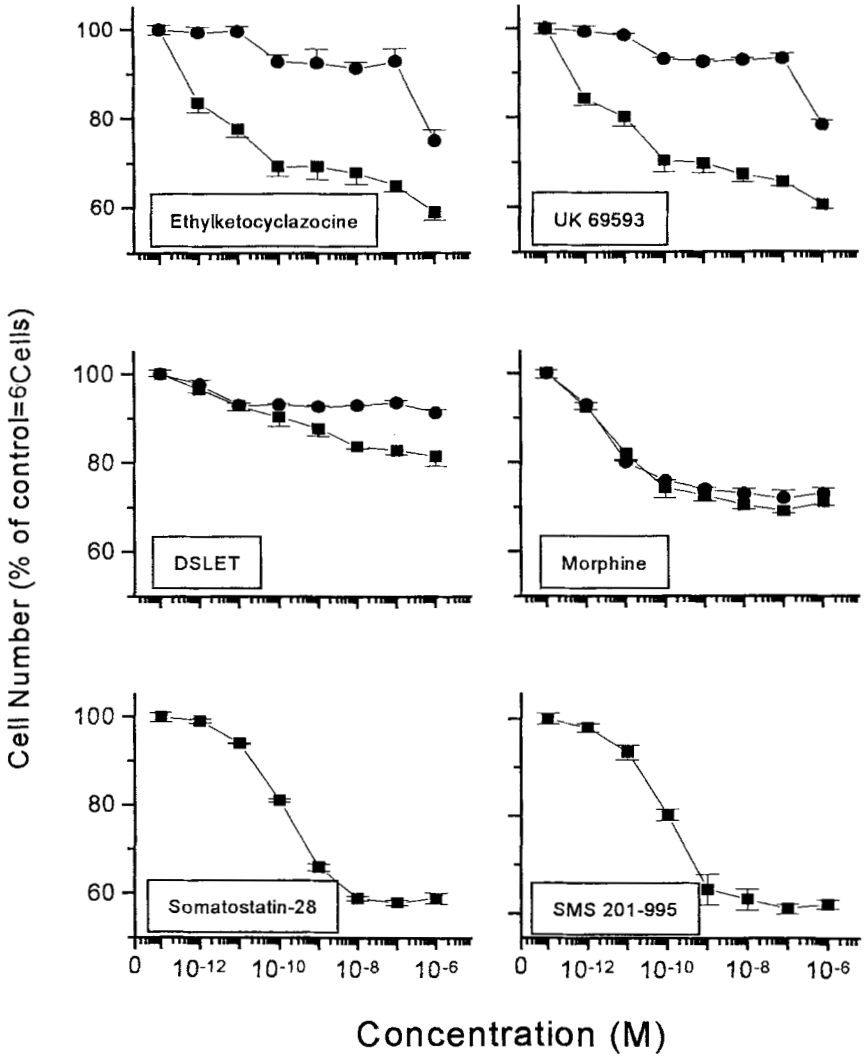

Fig. 4. Effect of opioid and somatostatin analogs on proliferation of opossum kidney (OK) cells. Twenty-five thousands cells were allowed to proliferate for 4 days (final density 100,000 cells) in the absence (control) or in the presence of the indicated concentrations of opioid agonists (ethylketocyclazocine, U69593, [D-Ser^2, Leu^5]enkephalin, Thr^6 (DSLET), and morphine) without (*squares*) or with (*circles*) the concominant addition of 10^{-6} M of the general opioid antagonist diprenorphine or of somatostatin-28 and sandostatin (SMS 201-995). The figure presents the number of cells as a percentage of control in the absence of any drug. Mean ± SE of three experiments in triplicate.

siderable homology [for reviews see Reisine and Bell, 1993, 1995; Reisine and Brownstein, 1995]. Furthermore, recent studies [Lachowicz et al., 1995; Orbuch et al., 1993] revealed new members of the seven-loop receptor family, which share mixed selectivities towards more than one class of ligands.

In view of the cited controversies concerning the presence of opioids and somatostatin in renal cells, what might be after all the physiological significance of the opioid and somatostatin binding described in the present study? Although the available information can allow only speculation, it seems possible that opioids and somatostatin could be produced during cell growth, either during ontogenesis or during tubular repair, modulating the proliferation of renal cells. Furthermore, as opioids and probably somatostatin could cross different barriers, it is plausible that they could reach the renal tubule and exert their effects on water excretion and/or cell proliferation.

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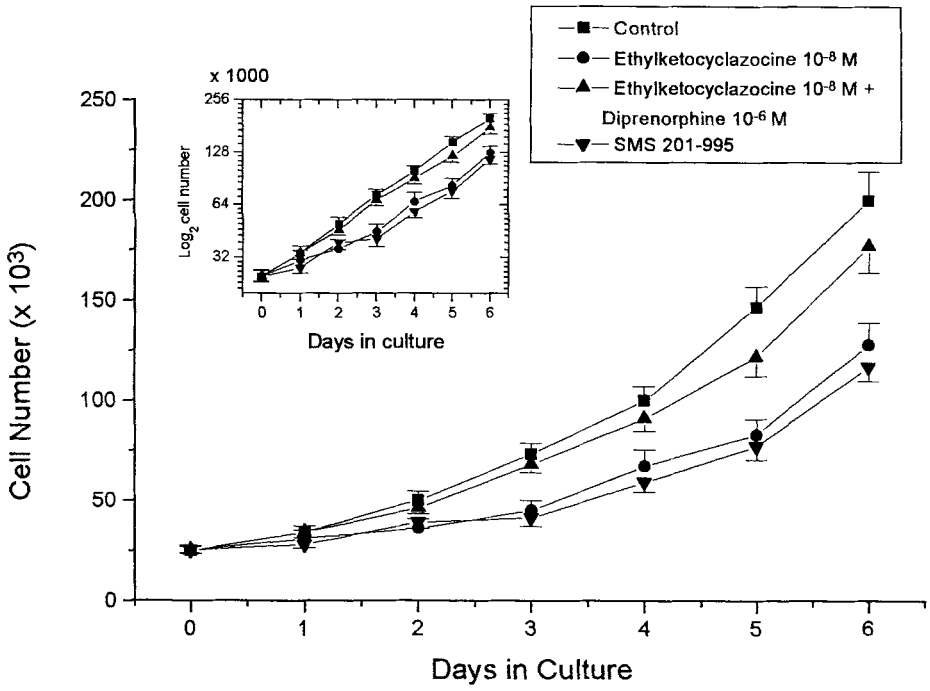

Fig. 5. Growth curve of OK cells in the presence of opioid or somatostatin analogs. Twenty-five thousand cells were cultured for the indicated time in the absence (*squares*) or in the presence (*circles*) of 10^{-8} M ethylketocyclazocine, 10^{-8} M ethylketocyclazocine plus 10^{-6} M diprenorphine (*up triangles*), or 10^{-8} M sandostatin (*down triangles*). At the indicated time intervals, cells were washed with phosphate-buffered saline, treated with trypsin, and directly counted. **Insert:** The same data in Log_2 coordinates.

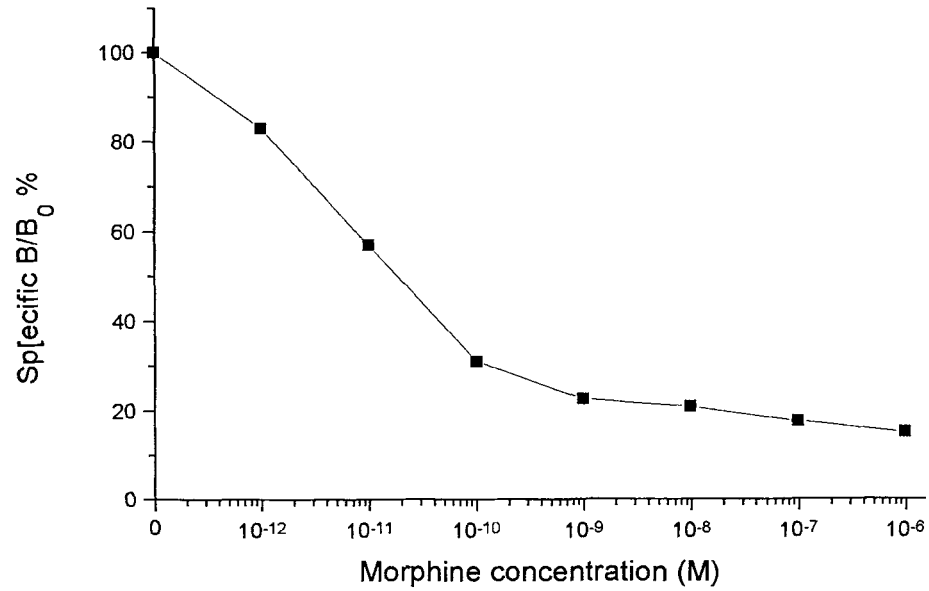

Fig. 6. Displacement of [^{125}I]Tyr^{11}-somatostatin-14 bound to OK cells by morphine [^{125}I]-Tyr^{11}-somatostatin (1.2 fmoles) (50,000 cpm) was incubated with the indicated concentrations of morphine. See Materials and Methods for details of the binding experiments. Mean ± SE of two separate experiments in triplicate.

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