Solubilization and Characterization of Active Neuropeptide Y Receptors from Rabbit Kidney*

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Active neuropeptide Y receptors were solubilized from rabbit kidney membranes using the zwitterionic detergent 3-[(3-cholamidopropyl)dimethylammonio]-1-propanesulfonic acid (CHAPS). In membrane fragments and soluble extracts neuropeptide Y binding was time dependent, saturable, reversible, and of high affinity. Scatchard analysis of equilibrium binding data indicated a single class of binding sites with respective K_D and B_{max} values of 0.09 nM and 530 fmol/mg of protein for the membrane-bound receptors and 0.10 nM and 1585 fmol/mg of protein for the soluble receptors. Neuropeptide Y binding was specifically inhibited by the nonhydrolyzable GTP analog guanosine 5'-O-(3-thiotriphosphate) in a concentration-dependent manner, with IC_{50} values of 28 and 0.14 µM for membrane-bound and soluble receptors, respectively, suggesting that neuropeptide Y receptors are functionally coupled to GTP-binding regulatory proteins. Cross-linking studies were performed with the heterobifunctional N-hydroxysuccinimidyl-4-azidobenzoate and the monofunctional neuropeptide Y derivative, azidobenzoyl and led to the identification of a 100 kDa peptide that should represent the covalently labeled neuropeptide Y receptor.

Neuropeptide Y (NPY)[1] is a 36-amino acid peptide structurally related to a group of brain-gut peptides, the so-called pancreatic polypeptide family. Although the other members of this family, pancreatic polypeptide and peptide YY, occur mainly in endocrine cells in the intestinal tract, neuropeptide Y is predominantly found in brain and the peripheral nervous system (1, 2). In sympathetic nerves it is co-localized and

released together with norepinephrine, with which it interacts both on a presynaptic and postsynaptic level (3–5). Physiological effects of neuropeptide Y resemble those elicited by noradrenergic stimulation. The peptide is an extremely potent constrictor of small blood vessels in many vascular beds and increases blood pressure in an adrenoreceptor-resistant manner when administered systematically (6). Injection into the brain stimulates food intake, decreases blood pressure, and causes neuroendocrine alterations. Peptide YY exerts quite similar effects, suggesting that both peptides can activate the same receptors (7).

Binding sites for neuropeptide Y have been described in numerous tissues including brain (8), spleen (9), aorta (10), and dorsal root ganglia (11). Although there is some indirect evidence that at least one type of neuropeptide Y receptor belongs to the class of G-protein coupled receptors (9, 12, 13), little is known about the structure and molecular properties of neuropeptide Y receptors.

We report here on some experiments aimed at characterizing neuropeptide Y binding sites on rabbit kidney membranes and present a protocol that allows solubilization of neuropeptide Y receptors in an active form. The rabbit kidney was chosen because this organ was recently reported to possess a particularly high density of high affinity binding sites for neuropeptide (Y) (14).

EXPERIMENTAL PROCEDURES

Materials—Neuropeptide Y was purchased from Bachem (Heidelberg, Federal Republic of Germany). Monoiodo ^{125}I-labeled Lys^4 neuropeptide Y and Na^{125}I, carrier-free, were purchased from Amersham Corp. The other chemicals were obtained from the following sources. N-Hydroxysuccinimidyl 4-azidobenzoate (NHSAB), CHAPS, phenylmethylsulfonyl fluoride, leupeptin, bacitracin, and the molecular weight markers for sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) were from Sigma; N,N'-dimethylformamide was from Merck; and chloramine T, bovine serum albumin, and all other chemicals, except stated otherwise, were purchased from Serva (Heidelberg, F. R. G.).

Preparation of Rabbit Kidney Membranes—Decapsulated rabbit kidneys (about 5 g each) were placed in 20 ml of homogenization buffer containing 50 mM Tris-HCl, pH 7.4, 5 mM MgCl$_2$, 1 mM EGTA, 1 mM phenylmethylsulfonyl fluoride, and 0.5% bacitracin. The tissue was finely minced with a polytron (setting 10) and homogenized with six strokes of a Teflon pestle in a tight fitting glass vessel. This homogenate was centrifuged for 10 min at 800 × g, the pellet discarded, and the supernatant centrifuged at 50,000 × g for 30 min in a Sorvall SS-34 rotor. The upper creamy layer of this pellet was gently removed, suspended in 20 ml of homogenization buffer, and recentrifuged at 50,000 × g for 30 min. The whole pellet was washed three times with homogenization buffer. The final membrane pellet was resuspended at a protein concentration of approximately 10 mg/ml homogenization buffer and shortly minced with a Polytron (setting 5). The designated membrane fragments were frozen in aliquots and stored at −20 °C.

Receptor Solubilization—The membrane fragments were thawed

[1] The abbreviations used are: NPY, neuropeptide Y; G-protein, GTP-binding protein; G$_i$, inhibitory GTP-binding regulatory component of adenylate cyclase; G$_o$, GTP-binding protein abundant in brain; GTPγS, guanosine 5'-O-(-3-thiotriphosphate); ATPγS, adenosine 5'-O-(3-thiotriphosphate); Gpp(NH)p, guanyl-5'-yl iminodiphosphate; NHSAB, N-hydroxysuccinimidyl 4-azidobenzoate; CHAPS, 3-[(3-cholamidopropyl)dimethylammonio]-1-propanesulfonic acid; EGTA, [ethylenebis(oxyethylenenitrilo)]tetraacetic acid; HPLC, high performance liquid chromatography; SDS, sodium dodecyl sulfate; PAGE, polyacrylamide gel electrophoresis; AB-NPY, azidobenzoyl neuropeptide Y; HEPES, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid.

and diluted in EGTA-free homogenization buffer containing various amounts of CHAPS in order to determine the optimal conditions for solubilization. After mixing gently for various times at 4 °C, the extract was ultracentrifuged at 150,000 × g for 1 h in a Beckman type TI-45 rotor. The supernatant was pressed through a 0.22-μm filter (Schleicher & Schüll) and is designated solubilized fraction.

Receptor Binding Studies with Membrane-bound and CHAPS-solubilized Receptors—All binding experiments were carried out at 0 or 25 °C in a total volume of 400 μl/tube for both the membrane fragments and the solubilized fraction.

The binding assay buffer contained 50 mM Tris-HCl, pH 7.4, 5 mM MgCl₂, 0.1% bovine serum albumin, 0.1% bacitracin, 0.1 mg/ml aprotinin, 0.1 mg/ml soybean trypsin inhibitor, and 10 μg/ml leupeptin. The solubilized fraction was adjusted to a final concentration of 3 mM CHAPS. The ligand binding to the membranes was terminated by rapid filtration through GF/C filters (Whatman) and three washes with 3 ml each of ice-cold buffer containing 50 mM Tris-HCl, pH 7.4, and 5 mM MgCl₂. The filters were dried, and the radioactivity was counted in a γ-spectrometer (multicrystal counter, Berthold, LB 2105). The binding of the solubilized fraction was stopped by the addition of 400 μl of an ice-cold dextran-coated charcoal suspension containing 3% Norit A (Serva) and 0.4% dextran in 50 mM Tris-HCl, pH 7.4, containing 5 mM MgCl₂. After 3 min on ice the tubes were centrifuged at 14,000 × g for 2 min, thus separating bound and free neuropeptide Y. The supernatants were decanted and measured for radioactivity in the spectrometer.

Nonspecific binding was determined by incubation in the presence of excess unlabeled neuropeptide Y (0.5 μM).

Saturation Experiments—Membrane fraction proteins and solubilized extracts were incubated for 60 min at 25 °C with increasing concentrations of a mixture of ¹²⁵I-neuropeptide Y and unlabeled neuropeptide Y (final concentration, 10–2500 pM). The binding parameters of the saturation curves were estimated using the nonlinear least squares curve-fitting program SCTFIT (15).

Kinetic Experiments—Membrane and solubilized fractions were incubated with ¹²⁵I-neuropeptide Y (0.4 nM). Association data were obtained by determining the specific binding at different times. At equilibrium of the reaction, dissociation was started by the addition of excess unlabeled neuropeptide Y (1 μM), and the time course of the specific binding was measured.

Preparation of Photolabile ¹²⁵I-Azidobenzoate Neuropeptide Y—The azidobenzoate derivative of neuropeptide Y was prepared by the following procedure. All steps with the photolabile reagent were handled in the dark.

Thirty μg of neuropeptide Y (7 nmol) in 30 μl of water was added to 30 μl of 50 mM HEPES, pH 8.0. To initiate the reaction, 7 nmol of NHSAB dissolved in 5 μl of dimethylformamide was added to the reaction mixture and incubated overnight at 4 °C. The reaction was quenched by the addition of 3 μl of 1 M Tris-HCl, pH 7.4, for 2 h at room temperature. The reaction products were separated in a reversed-phase HPLC system (Spectra Physics, Darmstadt) consisting of a 3.9 × 30-cm Delta Pak C-4 column (15-μm particles, 300 Å pore size, Millipore, Waters Chromatography Div., Eschborn, F. R. G.) as described (16). The column was equilibrated in 20% acetonitrile in 0.65% trifluoroacetic acid, and the elution was performed with an acetonitrile gradient of 20–40% over 50 min at a flow rate of 2 ml/min. The chromatography was followed by automatic recording of the absorbance at 220 nm. One-minute fractions were collected. The elution profile (Fig. 1) shows (without the injection peak) three major peak fractions that were lyophilized and analyzed for their ability to inhibit ¹²⁵I-neuropeptide Y binding to rabbit kidney membranes. Although the first peak represents unaltered neuropeptide Y, as could be demonstrated by equivalent retention times of neuropeptide Y in control HPLC runs under the same elution conditions, the two other peptides could be identified as neuropeptide Y derivatives in receptor binding studies. The neuropeptide Y molecule possesses two readily accessible amino groups for covalent attachment of the aminoactive ester group of NHSAB, Lys⁴ and the N-terminal Tyr¹. In accordance with this the two main reaction products should correspond to mono- and diazidobenzoyl derivatives of neuropeptide Y. The designated azidobenzoyl neuropeptide Y (AB-NPY) (see Fig. 1, fraction number 34) was iodinated by the chloramine-T procedure and was confirmed to be an azidobenzoyl derivative by its ability to photolabel rabbit kidney membranes.

For preparation of ¹²⁵I-azidobenzoyl neuropeptide Y, 5 μg of azidobenzoyl neuropeptide Y in a volume of 20 μl of 0.25 M NaP₁ buffer

metabisulfite (1 mg/ml). Free iodine was removed by chromatography on a C₁₈ Sep-Pak cartridge. Elution was performed stepwise with 10–100% methanol in 0.1% trifluoroacetic acid. The iodinated azidobenzoyl neuropeptide Y eluted at 80% methanol.

Affinity Labeling of Neuropeptide Y Binding Sites—Membrane fractions (100 μg/ml) were incubated with 100 pM ¹²⁵I-neuropeptide Y (2,000 Ci/mmol) in membrane binding assay buffer for 4 h at 0 °C. Membrane-bound labeled neuropeptide Y was separated by centrifugation at 14,000 × g for 15 min at 4 °C. The membranes were washed twice with ice-cold 50 mM HEPES, pH 7.4, containing 5 mM MgCl₂ and were finally resuspended in 300 μl of this buffer. The cross-linking reagent NHSAB was added to the membrane fragments at a final concentration of 1 mM (50 mM stock in dimethylformamide). After 5 min in darkness the samples with the photoreactive NHSAB were exposed to UV light (Mineralight TM-15) for 15 min at 0 °C. The reaction was then quenched by the addition of 20 μl of 3 M Tris, pH 6.8, and the membranes were pelleted by centrifugation at 14,000 × g for 10 min at 4 °C.

In studies with the photolabile neuropeptide Y derivative, the membranes were incubated with 0.4 nM ¹²⁵I-azidobenzoyl neuropeptide Y in a total volume of 400 μl of membrane binding assay buffer for 4 h at 0 °C in darkness. The membranes were centrifuged at 14,000 × g for 15 min at 4 °C and resuspended in 300 μl of 50 mM HEPES, pH 7.4, containing 5 mM MgCl₂. After irradiation with UV light for 15 min at 0 °C the samples were handled further as described above.

In competition experiments, membranes were incubated with ¹²⁵I-neuropeptide Y and increasing concentrations of unlabeled neuropeptide Y (10⁻⁷ to 10⁻¹¹ M). The pelleted membranes were analyzed by SDS-PAGE.

SDS-PAGE and Autoradiography—The membrane pellets were solubilized in sample buffer containing 125 mM Tris-HCl, pH 6.8, bromphenol blue, 10% (v/v) glycerol, and 2% (w/v) SDS in the presence or absence of 100 mM dithiothreitol. The samples were boiled for 3 min, and SDS-PAGE was performed according to Laemmli (17) with a 3.75% stacking and a 10% polyacrylamide resolving gel. After visualizing the proteins with Coomassie Blue R-250, the gels were destained and dried. Autoradiography was performed for 2–4 weeks at −70 °C on Kodak X-Omat AR films with Kodak X-Omatic regular intensifying screens.

Protein Determination—Protein concentrations in the membrane and soluble fractions were determined according to a modified Lowry method (18) using bovine serum albumin as a standard.

RESULTS

The Effect of Different CHAPS Concentrations on the Solubilization of Neuropeptide Y Binding Activity—Rabbit kidney membranes were treated for different times at 0 °C with various concentrations of the zwitterionic detergent CHAPS in order to determine the optimal conditions for solubilization of active neuropeptide Y receptors. The highest amount of specific neuropeptide Y binding activity with about 40% of the membrane proteins being solubilized was measured in the concentration range between 6 and 9 mM CHAPS. Although the total neuropeptide Y binding remained quite unchanged at higher CHAPS concentrations up to 20 mM, the nonspecific binding increased continuously parallel with a rising amount of solubilized membrane proteins. Incubation times longer than 40 min did not yield more receptor activity; therefore, solubilization with 8 mM CHAPS for 40 min at 0 °C was selected for all subsequent studies. The ¹²⁵I-neuropeptide Y binding activity present in both the membrane and solubilized fractions was stable for at least 10 days at 4 °C.

Equilibrium Binding of Neuropeptide Y to Membrane Preparations and Soluble Extracts—Saturation experiments with neuropeptide Y were performed in membrane fragments and CHAPS-solubilized fractions in order to determine and compare the binding parameters in both extracts (Fig. 2). In either case the specific binding of ¹²⁵I-neuropeptide Y was saturable, and their analyses by a nonlinear curve-fitting program (15) indicate a single class of high affinity binding sites, as shown

FIG. 1. **HPLC purification of AB-NPY.** Equimolar amounts of neuropeptide Y and NHSAB (7 nmol each) were allowed to react at 4 °C overnight in darkness (for details, see "Experimental Procedures"). The reaction mixture was quenched in 60 mM Tris-HCl, pH 7.4, for 2 h at room temperature and was applied to a C-4 column equilibrated in 20% acetonitrile in 0.065% trifluoroacetic acid. The elution was performed with an acetonitrile gradient of 20–40% (· · ·) over 50 min at a flow rate of 2 ml/min. One-minute fractions were collected, and the main peak fractions, detected by their absorbance at 220 nm, were lyophilized and tested in the receptor binding assay. Fraction 34, the designated AB-NPY, was iodinated by the chloramine-T procedure and further analyzed prior to its employment for photocross-linking.
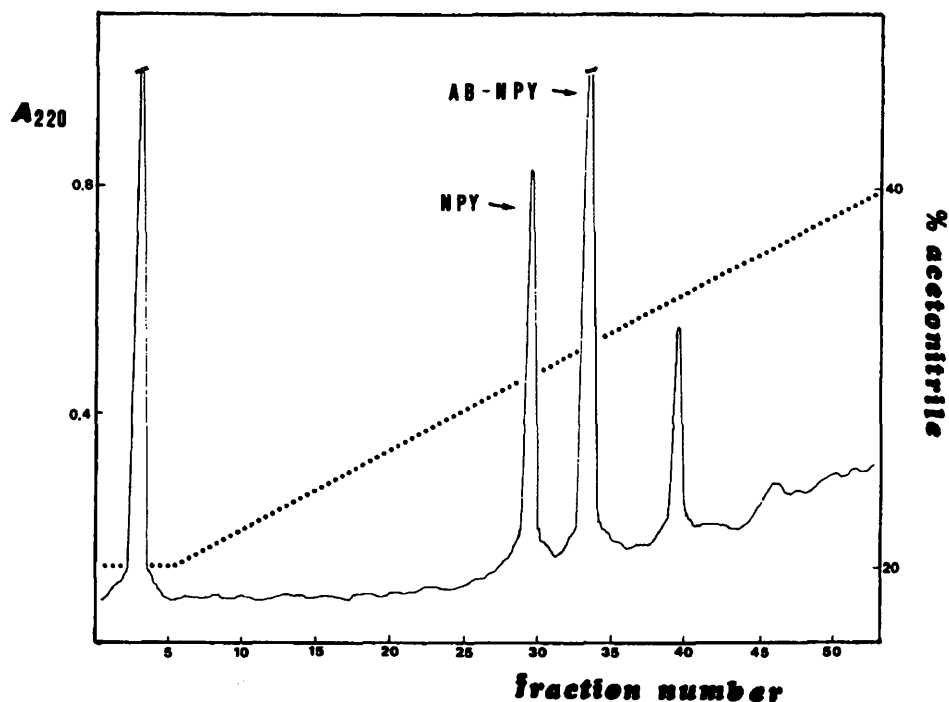
respective B_{max} and K_D values were 530 ± 70 fmol/mg of protein and 0.09 ± 0.02 nM for the membrane-bound receptors, and 1585 ± 130 fmol/mg of protein and 0.10 ± 0.02 nM for the CHAPS-solubilized receptors. Although unaltered dissociation values in both extracts demonstrate equal affinities of the receptors for neuropeptide Y, the higher number of binding sites (B_{max}) for the soluble fraction suggests an about 3-fold enrichment of neuropeptide Y receptors during the solubilization procedure. Nonspecific binding, expressed as per cent of total ligand bound at the K_D, decreased from about 25% for the membrane preparations to about 10% for the soluble extract, which might also be due to the effective separation of free and bound radioligand by dextran-coated charcoal adsorption in the case of the CHAPS-solubilized fraction.

Kinetics of ^{125}I-Neuropeptide Y Binding to Membranes and Soluble Fractions—Association and dissociation of neuropeptide Y binding to membranes and CHAPS-solubilized fractions were carried out at 25 °C (Fig. 3) and 0 °C. For both fractions binding was rapid, with 80% of maximal binding occurring within 10 min, and it reached a steady state at about 40 min. Neuropeptide Y binding remained stable over a period of at least 4 h at this temperature. At 0 °C half-maximal neuropeptide Y binding was obtained after about 40 min, and steady state was reached after 230 min (data not shown). After association to equilibrium, dissociation was initiated by the addition of 1 μM unlabeled neuropeptide Y. As shown in Fig. 3, neuropeptide Y binding was reversible but with a slow dissociation rate for both the membranes and the solubilized preparation. In order to test the possible role of G proteins in this process, we investigated the effect of guanine nucleotides on the time course of dissociation. The addition of the nonhydrolyzable GTPγS at a final concentration of 0.1 mM caused a dramatic increase of the dissociation rate. Within 5 min of incubation the specific ^{125}I-neuropeptide Y binding declined to about 30% for the membranes (Fig. 3A) and to less than 15% for the solubilized fraction (Fig. 3B). In control studies, ATPγS administered at an equal concentration of 0.1 mM did not alter neuropeptide Y binding at all (for

membranes) or only to a very slight extent (for soluble fractions).

Influence of Nucleotides on Neuropeptide Y Binding—Since we could demonstrate G-protein interaction with neuropeptide Y receptors for membrane-embedded as well as for CHAPS-solubilized binding sites in the previous dissociation study, we performed a dose-response relationship between neuropeptide Y binding and concentration of GTP and its stable analogs (10^{-8} to 10^{-3} M). As depicted in Fig. 4, neuropeptide Y binding decreased in the presence of GTPγS in a concentration-dependent manner, with an IC_{50} of about 28 μM GTPγS for the membranes (Fig. 4A) and 0.14 μM GTPγS for the soluble fraction (Fig. 4B), respectively. Experiments with Gpp(NH)p provided similar results; however, GTP was about 5–10-fold less potent (not shown). The inhibitory influence on neuropeptide Y binding to both fractions was guanine nucleotide specific, since ATP and its analogs caused far less pronounced effects.

Affinity Labeling of Neuropeptide Y Receptors—For further characterization of the neuropeptide Y receptors we performed cross-linking studies to rabbit kidney membranes with the heterobifunctional photoreactive NHSAB and with AB-NPY.

The azidobenzoyl neuropeptide Y was prepared as described under "Experimental Procedures." In competition experiments neuropeptide Y inhibited the binding of ^{125}I-azidobenzoyl neuropeptide Y to rabbit kidney membranes with an IC_{50} of 0.40 ± 0.08 nM (not shown). Nonspecific binding of this photolabile neuropeptide Y derivative was exceedingly high (about 70% of total ligand bound), a fact that might account for the numerous covalently labeled polypeptides obtained by photocross-linking ^{125}I-azidobenzoyl neuropeptide Y to rabbit kidney membranes (see Fig. 5A). However, when excess unlabeled neuropeptide Y (0.1 μM) was used for competition, one distinct band with a molecular mass of about 100 kDa was displaced (Fig. 5A, arrow).

Experiments with the heterobifunctional photolabile NHSAB resulted in substantial labeling of the 100-kDa complex. The dried gels were subjected to prolonged autoradiog-
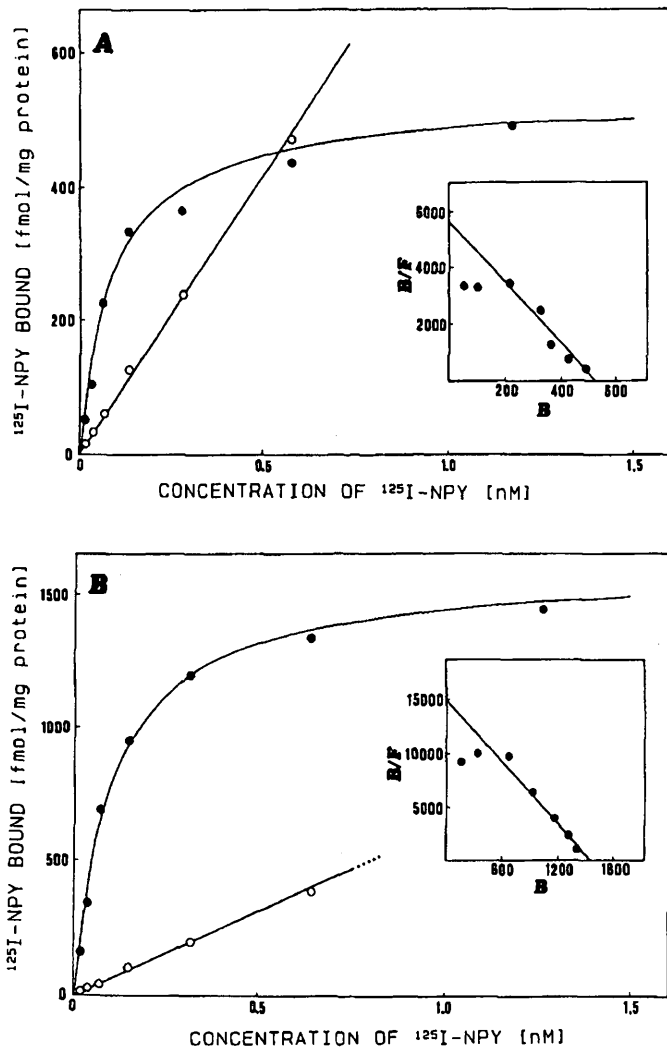
FIG. 2. **Saturation curves and Scatchard analyses of neuropeptide Y binding to membrane preparations (*panel A*) and to CHAPS-solubilized fractions (*panel B*).** Rabbit kidney membranes (35 μg/ml) and CHAPS-solubilized fractions (20 μg/ml) were incubated with increasing concentrations of ^{125}I-neuropeptide Y at 25 °C for 60 min. The bound radioactivity was separated from the free ligand by filtration for the membrane fragments and by dextran-coated charcoal adsorption for the soluble extract (for details, see "Experimental Procedures"). Nonspecific binding (O——O) was determined in the presence of 0.5 μM unlabeled neuropeptide Y. Specific binding (●——●) was estimated by subtracting nonspecific binding from total ligand bound. The *inset* shows the appropriate Scatchard plots: *B*, specifically bound neuropeptide Y in fmol/mg of protein; *F*, free neuropeptide Y in nM. Each point is an average of a triplicate determination. The binding parameters were fit using a nonlinear curve-fitting program (15).

raphy in order to expose nonspecific labeling, as illustrated in Fig. 5*B*.

Competition studies were performed for both cross-linkers and commonly showed displacement of the specifically labeled 100-kDa complex by unlabeled neuropeptide Y in the nanomolar concentration range, as demonstrated in Fig. 5*B*. There were no changes in the labeling pattern regardless of whether the cross-linking reactions were analyzed under reducing (100 mM dithiothreitol) or nonreducing conditions.

DISCUSSION

In the present study we demonstrate solubilization of neuropeptide Y receptors from rabbit kidney membranes in an

for the solubilization of numerous peptide receptors including those for angiotensin II (19), neurotensin (20), vasoactive intestinal peptide (21), and vasopressin (22). The soluble state of the receptors was confirmed by two experimental criteria. Neuropeptide Y binding activity passed through 0.22-μm filters and did not decrease following further ultracentrifugation at 150,000 × g for 60 min. The receptor characteristics remained unaltered during the solubilization procedure as was shown by saturation studies. In addition, neuropeptide Y binding to both membranes and soluble extracts was time dependent, saturable, and reversible. Although association of neuropeptide Y to its binding sites was quite rapid, dissociation occurred in a surprisingly slow fashion. However, the addition of GTP or its analogs accelerated drastically the dissociation of receptor-bound neuropeptide Y. This suggests that guanine nucleotides may regulate the binding of this peptide by triggering conversion of high into low affinity binding, as has been shown for other peptides (21, 23–25). The observations in the rabbit kidney are in agreement with those in membranes from rat brain (12) and pig spleen (9), in which regulation of neuropeptide Y binding by guanine nucleotides has been demonstrated.

Our observations indicate further that the soluble receptors are functionally coupled to G-proteins, either by reassociation of individually solubilized components or, alternatively, by acting as a whole and stable complex. The observation that for solubilized compared with membrane-embedded receptors ligand binding showed markedly enhanced sensitivity to guanine nucleotides has recently been reported also for somatostatin receptors (23).

It is not yet clear which of the various G-proteins so far described might serve as the signal transducer of neuropeptide Y binding in the kidney. Several studies have shown that the neuropeptide Y-induced effects are blocked by pretreatment with pertussis toxin. Among the G-proteins ribosylated by pertussis toxin, it is G_i that mediates ligand-induced inhibition of adenylate cyclase activity. Indeed, a reduction of cAMP accumulation by neuropeptide Y has been observed in many tissues including brain (26, 27), cerebral blood vessel (28), spleen (9), and a number of cell lines endowed with neuropeptide Y receptors (29). Thus, a G_i-protein could be associatd with the renal neuropeptide Y receptor. However, since recent observations in rat sensory neurons provided evidence for a G_o-induced coupling of neuropeptide Y receptors to calcium channels (30), it seems that more than one G-protein might function in the signal transduction pathway of this peptide.

In order to characterize further the neuropeptide Y receptor we performed cross-linking studies, thus identifying a 100-kDa peptide. Half-maximal inhibition of the labeling occurred with neuropeptide Y concentrations close to the K_D value, as obtained from saturation experiments. Therefore, we conclude that the 100-kDa polypeptide represents the covalently labeled neuropeptide Y receptor. Since reducing conditions did not change the labeling pattern, subunit structures caused by disulfide bridges appear not to be involved. The molecular size of the neuropeptide Y receptor described here compares well with those for some previously purified G-protein-associated peptide receptors determined by SDS-PAGE analysis (31–33). In contrast to our observations, studies on the cross-linking of neuropeptide Y to rat brain membranes, using two homobifunctional cross-linkers and NHSAB, resulted in specific labeling of two bands with respective molecular masses of 62 and 39 kDa (34). This discrepancy could reflect species-specific differences as has been described recently for chole-
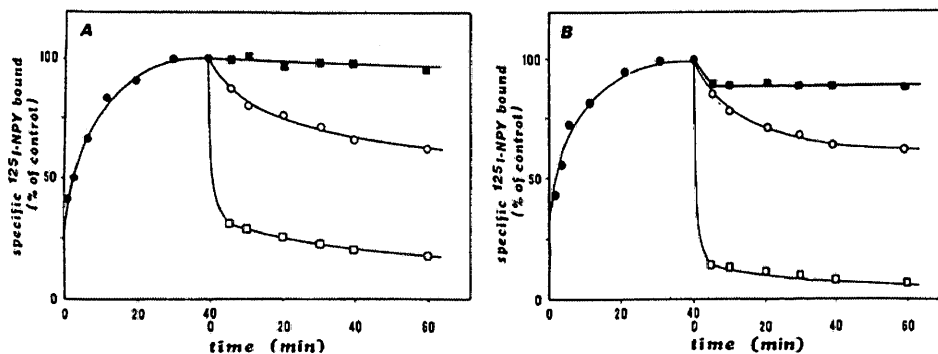
FIG. 3. **Kinetic experiments and effect of nucleotides on the dissociation of** [125]**I-neuropeptide Y bound to membranes** (*panel A*) **and soluble extracts** (*panel B*). Association kinetics (●——●) of ^{125}I-neuropeptide Y (0.4 nM) to rabbit kidney membranes (60 μg/ml) (*panel A*) and to CHAPS-solubilized extracts (20 μg/ml) (*panel B*) was examined at 25 °C. After 40 min, when equilibrium was reached, the dissociation (O——O) was started by the addition of excess unlabeled neuropeptide Y (final concentration, 1 μM). In parallel studies, GTPγS (final concentration, 0.1 mM) and unlabeled neuropeptide Y (final concentration, 1 μM) were added to both fractions, and the time course of dissociation was measured (□——□). In control experiments ATPγS was given at a final concentration of 0.1 mM after association to equlibrium, and the specific ^{125}I-neuropeptide Y binding was determined (■——■). All data are expressed as percentage of specific ^{125}I-neuropeptide Y binding at equilibrium and represent averages of two experiments performed in triplicate.
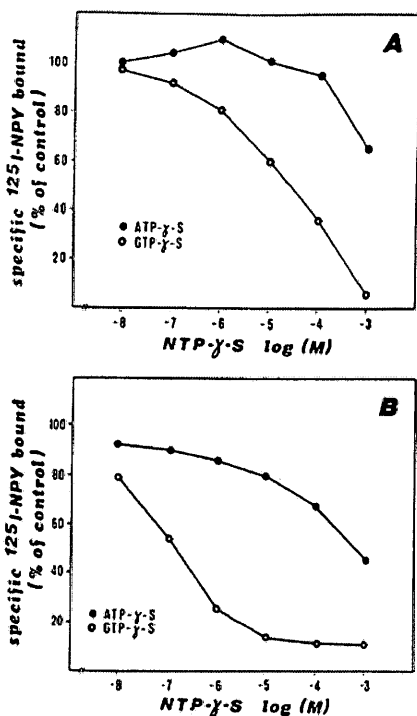

FIG. 4. **Effect of nucleotides on** [125]**I-neuropeptide Y binding to membrane-bound** (*panel A*) **and soluble receptors** (*panel B*). Rabbit kidney membranes (60 μg/ml) and CHAPS-solubilized fractions (20 μg/ml) were incubated with 0.4 nM ^{125}I-neuropeptide Y for 40 min at 25 °C in the absence or presence of increasing concentrations of the stable nucleotide analogs GTPγS (O——O) and ATPγS (●——●). Values are the means of a triplicate determination and are expressed as percent of neuropeptide Y binding obtained in the absence of the nucleotide triphosphates.

FIG. 5. **Photoaffinity labeling of neuropeptide Y binding sites.** Rabbit kidney membranes (100 μg/ml) were incubated to equilibrium with 0.1 nM ^{125}I-azidobenzoyl neuropeptide Y in the presence (+) or absence (−) of 0.1 μM unlabeled neuropeptide Y (*lanes A*) or with ^{125}I-neuropeptide Y and increasing concentrations of unlabeled neuropeptide Y (10^{-7} to 10^{-11} M) (*lanes B*) for 4 h at 0 °C. After washing and resuspension of the membranes in 50 mM HEPES, pH 7.4, containing 5 mM $MgCl_2$, cross-linking was initiated by exposure to UV light (*lanes A*) or by the addition of NHSAB at a final concentration of 1 mM, followed by irradiation (*lanes B*). All samples were allowed to react for 15 min on ice. The reaction was quenched by addition of Tris-HCl, pH 6.8 (final concentration, 170 mM), and the products were analyzed by SDS-PAGE under reducing conditions (100 mM dithiothreitol) prior to autoradiography.

more recent study concerned with the structural characterization of receptors for neuropeptide Y and peptide YY in various tissues including the rabbit kidney (37). In these experiments, cross-linking peptide YY to kidney membranes resulted in the labeling of two major proteins with respective molecular masses of 50 and 38 kDa in addition to another two specific but more faint bands in the range of 105 and 130 kDa. The employment of other cross-linking reagents as well as higher incubation temperatures with more risk of proteolytic degra-
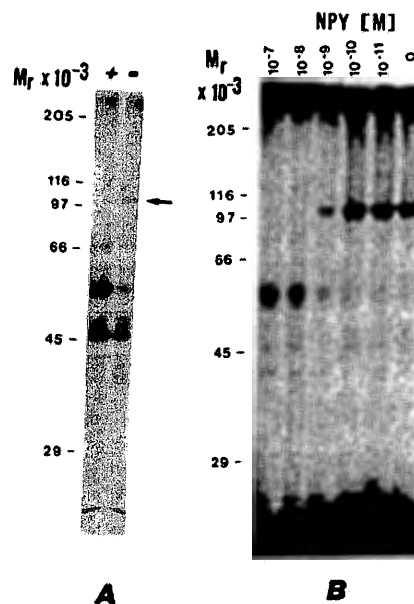
dation and the use of ^{125}I-Tyr[36] peptide YY in contrast to ^{125}I-Lys[4]-neuropeptide Y as the affinity ligand may explain the discrepancy between these and our results. It appears rather unlikely that the 100-kDa protein described here represents an artifactual aggregate of the receptor with another protein such as components of G_i or G_o, since the same protein was identified whether the monofunctional neuropeptide Y derivative or the bifunctional cross-linker NHSAB was used.

The identification of receptors for neuropeptide Y and

peptide YY in the kidney argues for a role of peptides of the pancreatic polypeptide family in the control of kidney function. In fact, neuropeptide Y has been shown to induce renal vasoconstriction and to attenuate renin release from the kidney (38, 39). In addition, studies in the isolated perfused rat kidney suggested that neuropeptide Y has also some natriuretic activity (39). This is of particular interest with respect to the distribution of neuropeptide Y binding sites within the kidney. According to autoradiographic studies in the rabbit kidney, neuropeptide Y binding is not confined to the vasculature, but can also be observed over the proximal convoluted tubuli (40). It is therefore intriguing to speculate that the cortical neuropeptide Y receptors we have characterized here play some part in the mediation of the tubular effects of neuropeptide Y.

In summary, in this paper we demonstrate the solubilization of neuropeptide Y receptors from rabbit kidney in an active form using the detergent CHAPS. Neuropeptide Y binding to membrane-embedded and soluble receptors was sensitive to guanine nucleotides, suggesting a G-protein-coupled receptor type. Cross-linking studies revealed a 100-kDa covalently labeled receptor complex. These observations should facilitate future studies directed at the purification and molecular characterization of the neuropeptide Y receptor.

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