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Characterization of tachykinin NK2 receptor in the anterior pituitary gland

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Abstract

Tachykinins are a family of bioactive peptides that interact with three subtypes of receptors: NK1, NK2 and NK3. Substance P has greater affinity for NK1, and neurokinin A (NKA) for NK2 receptor subtype. Although only NK1 receptor has been characterized in the anterior pituitary gland, some evidence suggests the existence of NK2 receptors in this gland. Therefore, we investigated the presence of NK2 receptors in the anterior pituitary gland of male rats by radioligand binding studies using labeled SR48968, a non peptidic specific antagonist. [^{3}H]SR48968 specific binding to cultured anterior pituitary cells was time-dependent and saturable, but with a lower affinity than previously reported values for cells expressing NK2 receptors. Unlabeled NKA inhibited only partially [^{3}H]SR48968 specific binding to whole anterior pituitary cells. Since SR48968 is a non polar molecule, we performed experiments to discriminate surface from intracellular binding sites. SR48968 exhibited both surface and intracellular specific binding. Analysis of the surface-bound ligand indicated that [^{3}H]SR48968 binds to one class of receptor with high affinity. Neurokinin A completely displaced [^{3}H]SR48968 surface specific binding fitting to a two-site/two-state model with high and low affinity. Additionally, immunocytochemical studies showed that the NK2 receptor is expressed at least in a subset of lactotropes. These results demonstrate the presence of NK2 receptors in the anterior pituitary gland and suggest that NKA actions in this gland are mediated, at least in part, by the NK2 receptor subtype.
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Keywords: NK2 receptor; Neurokinin A; SR48968; Anterior pituitary; Tachykinins

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Introduction

Tachykinins are a family of bioactive peptides that includes substance P (SP), neurokinin A (NKA), neuropeptide K (NPK), neuropeptide γ (NPγ) and neurokinin B (NKB). They are involved in a variety of physiological processes such as nociception, neurogenic inflammation, broncoconstriction, nigrostriatal motor activity and immunomodulation (Otsuka and Yoshioka, 1993; Lecci et al., 2000).

These peptides exert their effects interacting with three subtypes of G protein-linked receptors: NK1, NK2 and NK3. Substance P has greater affinity for the NK1 receptor than for the other NK receptor subtypes; NKA, NPK and NPγ for NK2, and NKB for NK3 receptor. However, tachykinins show poor selectivity interacting with the three receptor subtypes as full agonists (Regoli et al., 1994).

The presence of SP and NKA in the median eminence and in the anterior and neurointermediate lobes of the pituitary gland has suggested that these peptides participate in the control of pituitary hormone secretion (Larsen et al., 1992; Duval et al., 1996; Brown et al., 1991). Substance P stimulates in vitro prolactin release from anterior pituitary explants and LH secretion from cultured anterior pituitary cells of adult female rats (Vijayan and McCann, 1979; Shamgochian and Leeman, 1992). NK2 receptor agonists such as NPK and NPγ also increase LH release (Kalra et al., 1992).

We have observed that the immunoneutralization of endogenous NKA reduces the proestrus prolactin surge in cycling female rats and the hyperprolactinemia induced by suckling in lactating rats (Pisera et al., 1991, 1998). This stimulatory effect of NKA on prolactin release is exerted, at least in part, at the pituitary level, since this peptide stimulates prolactin secretion from incubated anterior pituitaries of male rats (Pisera et al., 1994). In female rats, the direct effect of NKA on prolactin release is modulated by gonadal steroids. Neurokinin A increases in vitro prolactin release from incubated hemipituitaries from rats in proestrus and estrus, but not in diestrus or from ovariectomizad rats (Pisera et al., 1998).

Binding studies have shown that NK1 receptors are present in the anterior pituitary gland (Larsen et al., 1989a,b). The number of anterior pituitary SP-binding sites changes during the estrus cycle reaching the highest level at proestrus (Kerdelhué et al., 1985). Kalra et al. have suggested that the effect of NPK on the in vitro LH release may be mediated by pituitary NK2 receptors (Kalra et al., 1992). In fact, the secretory response of gonadotropes to LHRH is reduced by NK2 receptor antagonists (Debeljuk et al., 1997). Also, we have observed that the stimulatory effect of NKA on prolactin secretion from cultured anterior pituitary cells is blocked by a specific NK2 receptor antagonist (Pisera et al., 1998). Although these findings strongly suggest that the NK2 receptor is expressed in the anterior pituitary gland, this tachykinin receptor subtype has not yet been characterized in the anterior pituitary.

In the present work we investigated the expression of the NK2 receptor subtype in the anterior pituitary of male rats by radioligand binding assays, using SR48968 (a non peptidic specific NK2 receptor antagonist) as labeled ligand (Emonds-Alt et al., 1992). Additionally, we performed immunocytochemical studies to evaluate the presence of NK2 receptor in lactotropes.

Materials and methods

All drugs, media and supplements were obtained from Sigma Chemical Co. (St Louis, MO, USA) except fetal bovine serum (FBS) (GenSa, Buenos Aires, Argentina), [^{3}H]SR48968 (NEN Life Science Products, Inc., MA, USA), $MnCl_2$ (ICN Biomedicals, OH, USA), Solvable (Packard Instrument Co, CT, USA), donkey anti-guinea pig secondary antibody (Chemicon Int. CA, USA) and NKA (Peninsula Lab.

Inc., CA, USA). Unlabeled SR48968 was kindly supplied by Dr. X. Emonds-Alt, Sanofi Recherche, France.

Animals

Adult male Wistar rats weighing 150–200 g were used. The animals were housed in a light (12 h light/dark cycle) and temperature-controlled environment with food and water ad libitum, and treated following the NIH Guide for the Care and Use of Laboratory Animals. The rats were killed by decapitation.

Anterior pituitary cell culture

The pituitary gland was removed within minutes after decapitation and the neurointermediate lobe detached. The anterior pituitary glands were washed several times with Dulbecco's Modified Eagle's medium (DMEM) and cut into small fragments. The slices were dispersed enzymatically by successive incubations in DMEM supplemented with 3 mg/ml bovine serum albumin (BSA), containing 2.5 mg/ml trypsin (Type I from bovine pancreas), 1 mg/ml DNase (Deoxyribonuclease II, Type V from bovine spleeen), and 1 mg/ml trypsin inhibitor (Type II-S from soybean). The cells were finally dispersed mechanically by extrusion through a Pasteur pipette in Krebs buffer (KRB) without Ca^{++} and Mg^{++}. Dispersed cells were washed twice and suspended in DMEM supplemented with 10 μl/ml MEM aminoacids, 5.6 μg/ml amphotericin B, 25 μg/ml gentamicin and 2 mM glutamine (DMEM-S). Cell viability, assessed by trypan blue exclusion, was above 90%. The cells were seeded onto 96- or 48-well culture plates at different cell densities and cultured in DMEM-S with 10% FBS for 2–3 days in a humidified atmosphere of 5% CO_2–95% air at 37 °C.

Binding experiments

Cultured cells

Cultured anterior pituitary cells were washed three times in a medium containing 118 mM NaCl, 4.7 mM KCl, 2.54 mM $CaCl_2$, 1.18 mM $MgSO_4$, 3 mM $MnCl_2$, 12.44 mM $NaHCO_3$, 10 mM HEPES, 10 mM glucose, 0.02% BSA, pH 7.4, and incubated in 250 μl of the same medium plus protease inhibitors (40 μg/ml bacitracin, 10 μg/ml aprotinin, 5 μg/ml chymostatin and 5 μg/ml leupeptin) at 20 °C in a 95% O_2–5% CO_2 atmosphere. Incubations were performed in the presence of [^{3}H]SR48968, with different concentrations of unlabeled SR48968 or NKA for different time periods according to the experimental design. After the incubation, the cells were washed three times and lysed with 2 N NaOH. In some experiments, surface-bound ligand was previously released by exposing cells to hypertonic acid buffer (0.5 M NaCl, 0.2 M acetic acid, pH 3.0) for 5 min (Nouel et al., 1997). Radioactivity in aliquots of these media was measured in a scintillation counter.

Anterior pituitary membrane suspension

Forty anterior pituitaries of male rats were homogenized in 15–20 vol of 0.25 M sucrose-20 mM Tris buffer (pH 7.3) and centrifuged at 400 rpm for 5 min. The supernatant was centrifuged at 400 rpm for 5 min, and then at 10000 rpm for 20 min.The pellet was resuspended in 15 vol of the same buffer and centrifuged at 10000 rpm for 20 min. The resulting pellet was resuspended in binding

buffer (50 mM Tris, 150 mM NaCl, 3 mM $MnCl_2$, 0.1% BSA, 100 μg/ml bacitracin, 10 μg/ml aprotinin, 10 μg/ml chymostatin and 5 μg/ml leupeptin, pH 7.3) and stored at − 70°C until used. An aliquot was taken for protein determination by the method of Lowry et al. (1951). Membrane suspension was diluted in binding buffer (0.6 mg of protein/ml) and incubated at 20 °C in a 95% O_2–5% CO_2 atmosphere with shaking, in the presence of [^{3}H]SR48968 and different concentrations of unlabeled SR48968 or NKA. After competition experiments, bound radioactivity was separated by centrifugation at 10000 rpm for 20 min. Pellets were resuspended in Solvable and radioactivity was measured in a scintillation counter.

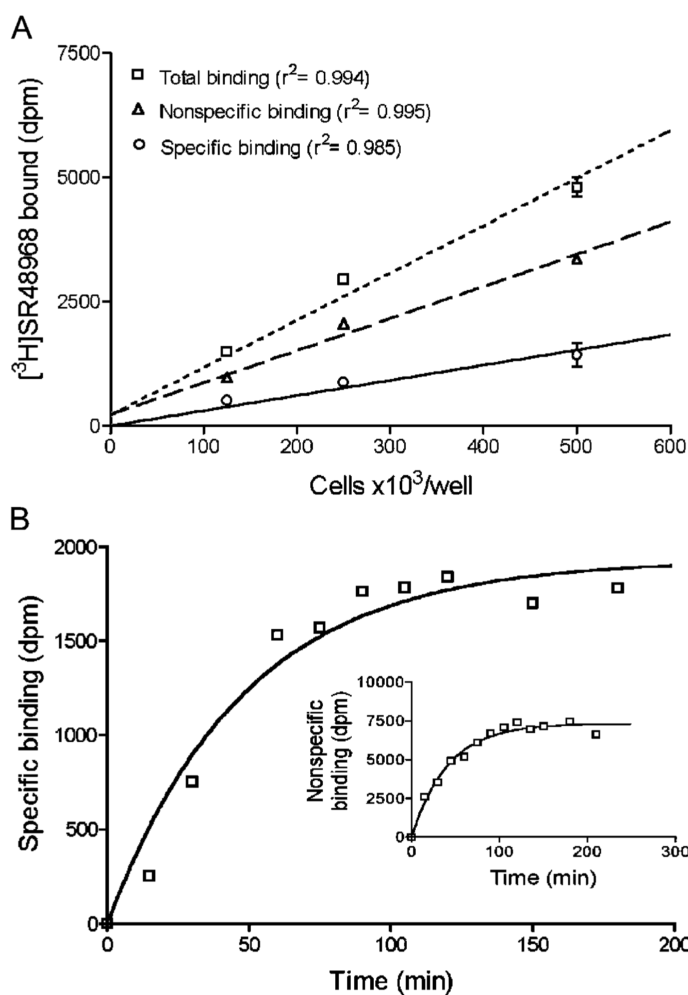

Fig. 1. Specific binding of [^{3}H]SR48968 increased linearly with cell number and in a time-dependent manner. A. Anterior pituitary cells were incubated at different densities with 1 nM [^{3}H]SR48968 for 180 min. Nonspecific binding was determined in the presence of 2 μM unlabeled SR48968. B. 250000 anterior pituitary cells/well were incubated with 1.44 nM [^{3}H]SR48968 for 15–180 min. Inset: Nonspecific binding of [^{3}H]SR48968 determined in the presence of 2 μM unlabeled SR48968.

Analysis of binding data

All experiments were performed at least twice. Figures represent results of individual experiments and data are the mean of triplicate incubations. Curve fitting and binding parameters were obtained by nonlinear regression analysis. In addition, surface binding data were linearly transformed according to Scatchard to allow an easier examination of the curves (Renzetti et al., 1997). Linear and nonlinear regression analysis were done with Prism 2 (GraphPad Software, Inc., San Diego, CA).

Immunocytochemistry

Anterior pituitary expression of NK2 receptor subtype was studied by double labeled indirect immunofluorescence. After 3 days of culture, anterior pituitary cells were washed three times with 0.1 M phosphate-buffered saline (PBS) pH 7.4 and fixed in 4% formaldehyde in PBS at 4 °C for 30 min. Nonspecific binding was blocked by incubation with 5% sheep serum and 5% donkey serum in 0.2% Triton X-100 in PBS for 45 min at room temperature. Slides were incubated overnight at 4 °C with primary antibodies: guinea pig anti-rat prolactin (NHPP-IC, 1:2500) and rabbit anti-NK2 receptor (1:500), diluted in 0.2% Triton X-100 containing 0.5% sheep serum and 0.5% donkey serum. After rinsing, slides were incubated for 1 h at room temperature with secondary antibodies: donkey anti-guinea pig conjugated to rhodamine and sheep anti-rabbit conjugated to fluorescein isothiocyanate, at 1:200 dilution in the same buffer. Then, coverslides were mounted with 1 μg/ml DAPI (4′ ,6-diamidino-2-phenyindole) in 90% glycerol solution containing 2.33% DABCO (1,4-diazabicyclo[2.2.2]octane). Control slides were incubated either with neutralized anti-NK2 antiserum by preincubation overnight at 4 °C with 20 μg/ml of antigenic peptide ($NK2_{376-390}$ C-terminal fragment) or in the absence of primary antibodies. The anti-NK2 receptor antibody and the antigenic peptide were obtained by E. F.

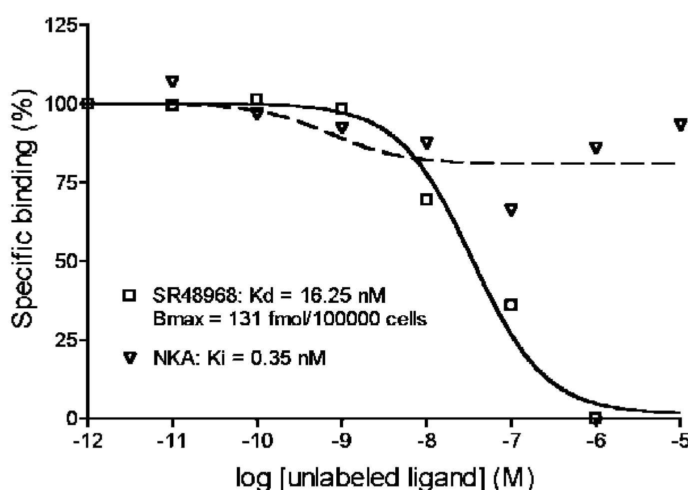

Fig. 2. Binding of [^{3}H]SR48968 to whole anterior pituitary cells was inhibited by unlabeled SR48968 or NKA. Anterior pituitary cells (250000 cells/well) were incubated with 1.12 nM [^{3}H]SR48968 and increasing concentrations of unlabeled SR48968 or NKA for 120 min. Nonspecific binding was determined in the presence of 10 μM of unlabeled SR48968. Results are expressed as percentage of specific binding in the absence of SR48968 or NKA.

Grady, University of California, San Francisco (Grady et al., 1996), and kindly supplied by Dr. J. C. Marvizón, University of California, Los Angeles.

Results

Binding of SR48968 to anterior pituitary cells

Fig. 1A shows that [^{3}H]SR48968 bound specifically to cultured anterior pituitary cells of male rats. Total and specific binding of [^{3}H]SR48968 increased linearly with cell number. In order to achieve radioligand binding lower than 10% of total added radioligand, the following experiments were

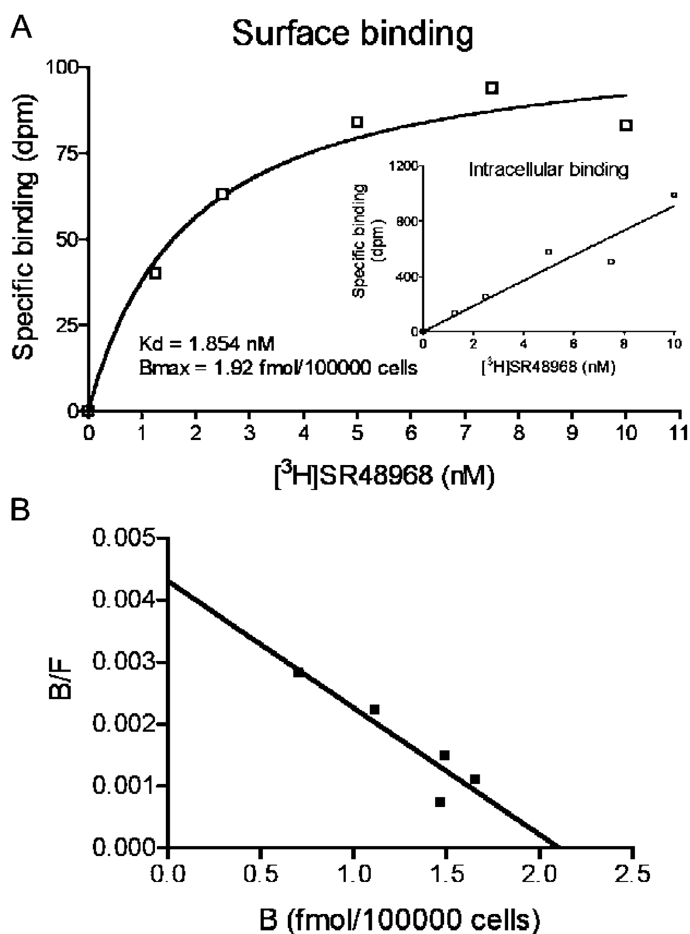

Fig. 3. Saturation analysis of surface-binding of [^{3}H]SR48968 to anterior pituitary cells. Anterior pituitary cells (100000 cells/well) were incubated with increasing concentrations of [^{3}H]SR48968 for 120 min. Nonspecific binding was determined in the presence of 2 μM unlabeled SR48968. Surface-bound ligand was released as described in Materials and methods. A. Nonlinear regression analysis. B. Scatchard plot.

performed with 100000–250000 cells/well. In this condition, [^{3}H]SR48968 specific binding to anterior pituitary cells was about 35% of total binding.

[^{3}H]SR48968 specific binding increased in a time-dependent manner reaching equilibrium after 100 min of incubation and remaining stable for at least another 80 min (Fig. 1B).

Equilibrium binding studies

When anterior pituitary cells were incubated with increasing concentrations (0.1–60 nM) of [^{3}H]SR48968 for 120 min, data analysis showed that [^{3}H]SR48968 specific binding was saturable with a Kd = 16.78 nM and a Bmax = 78 fmol/100000 cells. In competition studies, [^{3}H]SR48968 specific binding was inhibited by increasing concentrations of either unlabeled SR48968 or NKA (Fig. 2). Although NKA showed higher affinity than unlabeled SR48968, 10^{-6} M NKA inhibited only about 25% of [^{3}H]SR48968 specific binding, suggesting the presence of non-NK2 specific binding sites for SR48968.

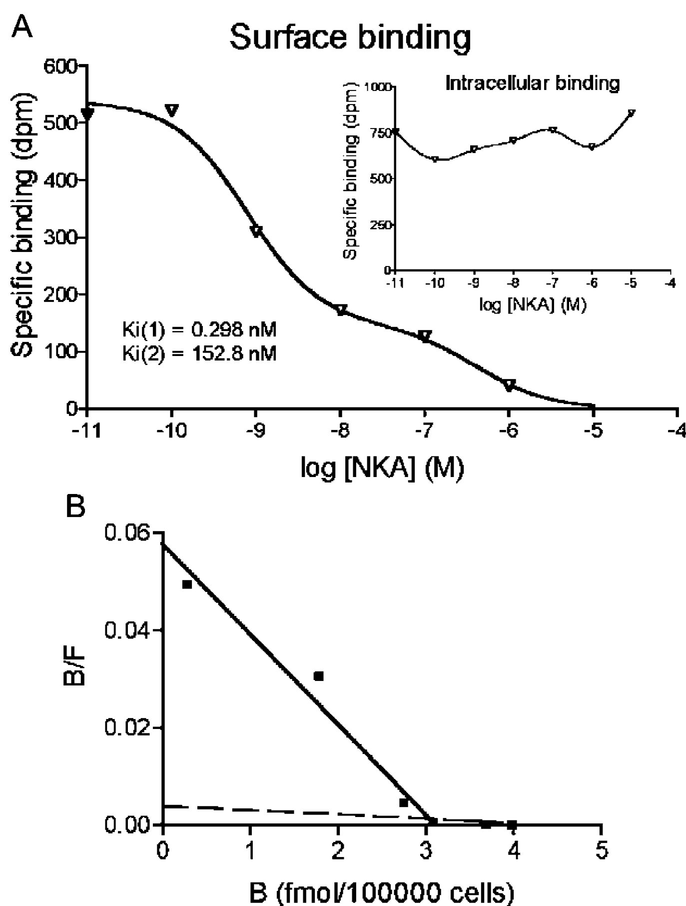

Fig. 4. Neurokinin A inhibits [^{3}H]SR48968 surface binding to anterior pituitary cells in a concentration dependent manner. Anterior pituitary cells (250000 cells/well) were incubated with 3 nM [^{3}H]SR48968 and increasing concentrations of NKA for 120 min. Nonspecific binding was determined in the presence of 10 μM unlabeled SR48968. Surface-bound ligand was released as described in Materials and methods. A. Nonlinear regression analysis. B. Scatchard plot.

Differentiation of surface from intracellular [^{3}H]SR48968 binding sites

In view of the nonpolar nature of SR48968, we performed additional experiments to determine the existence of intracellular binding sites for this ligand. To avoid binding to cytosolic proteins, we ran competition experiments using a suspension of membranes prepared from anterior pituitary glands instead of whole cells. The membrane suspension was incubated with 3 nM of [^{3}H]SR48968 and increasing concentrations of unlabeled SR48968 or NKA for 100 min. [^{3}H]SR48968 specific binding to

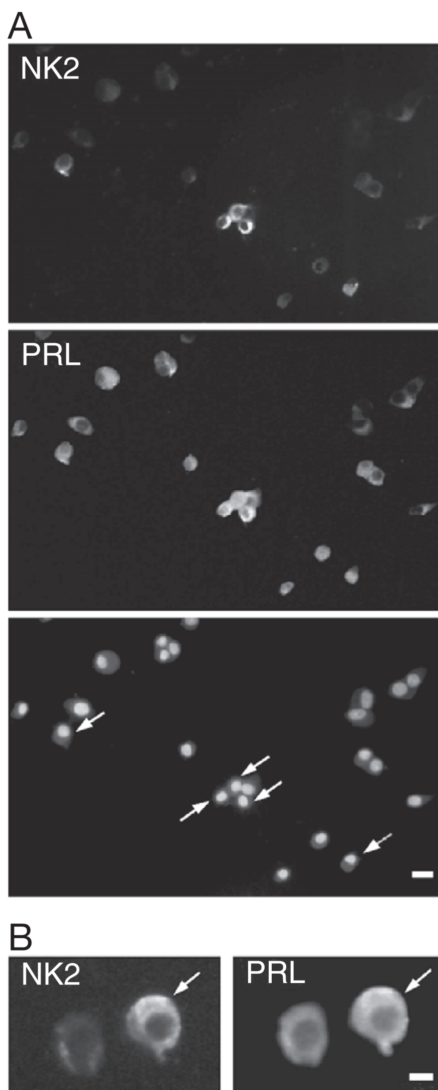

Fig. 5. Presence of NK2 receptors in lactotropes. A. Immunofluorescent detection of NK2 receptors in primary anterior pituitary cultures. Upper panel shows immunopositive cells for NK2 receptors and middle panel for prolactin. Arrows in lower panel indicate nuclei of double labeled cells counterstained with DAPI (scale bar: 25 μm). B. Arrows indicate a lactotrope showing NK2-immunoreactivity (scale bar: 10 μm).

anterior pituitary membranes was about 40% of total binding and was completely inhibited by SR48968 (Kd = 0.38 nM) or NKA (Ki = 0.48 nM). These competition curves indicated that both ligands bind to high affinity sites in anterior pituitary membranes, suggesting that in whole cells SR48968 binds to both surface NK2 receptors and non-NK2 intracellular binding sites.

To confirm the presence of intracellular specific binding sites for SR48968, we performed saturation and displacement binding studies in whole anterior pituitary cells, differentiating surface-bound from intracellular-bound radioactivity. These experiments showed the existence of specific [^{3}H]SR48968 binding sites in both compartments (Fig. 3 and Fig. 4). Saturation analysis of surface-bound ligand indicated that [^{3}H]SR48968 binds to one class of receptor with high affinity (Fig. 3). At the assayed concentrations of [^{3}H]SR48968, intracellular specific binding did not reach saturation (Fig. 3A, inset). NKA inhibited [^{3}H]SR48968 surface binding to anterior pituitary cells in a concentration dependent manner (Fig. 4). Analysis of the displacement curve indicated that NKA binds to high and low affinity sites, which comprise 73% and 27% of surface binding sites, respectively. Intracellular [^{3}H]SR48968 specific binding was not inhibited by NKA (Fig. 4A, inset).

Localization of NK2 receptors

Immunocytochemical studies showed that the majority of the NK2 immunoreactive cells were lactotropes. Only a subset of lactotropes expressed NK2 receptor subtype (Fig. 5).

Discussion

The present study demonstrates the presence of the NK2 tachykinin receptor subtype in anterior pituitary cells. We previously suggested that the stimulatory effects of NKA on prolactin release from anterior pituitary cells is mediated by its interaction with NK2 receptors. Specifically, we observed that the effect of NKA on prolactin release was blocked by L-659,877, a specific NK2 receptor antagonist (Pisera et al., 1998).

Previous competition studies using iodinated SP as labeled ligand indicated a single binding site for this tachykinin in the anterior pituitary that was characterized as the NK1 receptor (Larsen et al., 1989a,b). However, the low concentration of labeled ligand used in these reports and the relatively low affinity of SP for NK2 receptor (Regoli et al., 1989) could have prevented the detection of this tachykinin receptor subtype.

Naturally occurring agonists were frequently used as labeled ligands for identification of tachykinin receptor subtypes by binding assays. However, these peptides are preferential rather than selective ligands for the tachykinin receptor subtypes (Regoli et al., 1994). Also, tachykinins are very susceptible to the action of proteases (Larsen et al., 1989b; McKee et al., 1993). These difficulties for the accurate identification of receptor subtypes have been partially solved by the introduction of non peptidic tachykinin receptor antagonists, such as SR48968. SR48968 is accepted to bind specifically to NK2 receptor (Regoli et al., 1994). This compound has been shown to inhibit the binding of [^{125}I]NKA to rat duodenum membranes with high affinity (Emonds-Alt et al., 1992). On the contrary, SR48968 does not inhibit (Ki>5000 nM) the binding of NK1 or NK3 specific ligands (Emonds-Alt et al., 1992). In addition, the use of receptor antagonists for binding studies in living cells avoids many of the events induced by receptor agonist binding such as conformational changes in the

receptor protein, its interaction with G protein and ligand-receptor complex internalization (Renzetti et al., 1997).

Our study shows that $[^3H]$SR48968 binds specifically to anterior pituitary cells and that this binding is time dependent and saturable. However, analysis of binding data to whole anterior pituitary cells showed higher Kd values for SR48968 than those previously reported in other tissues or in cell lines expressing NK2 receptors (Renzetti et al., 1997; Huang et al., 1995). The relative high Kd values for SR48968, a nonpolar molecule, plus the fact that $[^3H]$SR48968 binding inhibition by NKA was partial, suggested the presence of additional intracellular binding sites for this antagonist in anterior pituitary cells. This hypothesis was investigated by separating surface from intracellular $[^3H]$SR48968 binding. When displacement experiments were performed in membrane suspensions, both unlabeled SR48968 and NKA completely inhibited $[^3H]$SR48968 specific binding showing Ki values below 1 nM. Surface binding of $[^3H]$SR48968 in anterior pituitary cells was saturable showing a Kd value similar to those reported by others (Huang et al., 1995). Surface $[^3H]$SR48968 specific binding was displaced by NKA fitting to a two-site/two-state model ($p = 0.015$) that may represent G protein-coupled (high affinity) and G protein-uncoupled (low affinity) receptors as it was previously reported for NK2 receptors in other systems (Renzetti et al., 1997; Huang et al., 1995). Altogether, these observations strongly support the presence of NK2 receptors in anterior pituitary cells.

Although SR48968 has been reported to bind to voltage-dependent Ca^{++} channels, its affinity for these channels is low (Ki value about 3 μM) (Lombet and Spedding, 1994). The high affinity binding of SR48968 in surface binding experiments and the complete inhibition of this binding induced by NKA rule out the presence of non-NK2 surface specific binding sites for SR48968 in the anterior pituitary.

Previous studies showed that substance P is present in subsets of somatotropes and thyrotropes (Brown et al., 1991). Because substance P and NKA are mostly cosynthesized, it is possible to speculate that this tachykinin is also present in these cell types (Debeljuk and Lasaga, 1999). Our immunocytochemical experiments show that the NK2 receptor subtype is expressed in lactotropes. NKA may be released from somatotropes and thyrotropes exerting a paracrine effect on lactotropes through interaction with NK2 receptors. In fact, the in vitro neutralization of endogenous NKA by addition of an anti-NKA serum to the incubation medium inhibits basal prolactin release (Pisera et al., 1994), and the blockade of NK2 receptors by SR48968 induces a decrease in anterior pituitary prolactin secretion (Debeljuk et al., 1997).

Conclusion

Our observations evidence the presence of the NK2 tachykinin receptor subtype in anterior pituitary cells of male rats, particularly in lactotropes. This receptor subtype may be involved in NKA actions on pituitary hormone secretion.

Acknowledgements

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References

Brown, E.R., Roth, K.A., Krause, J.E., 1991. Sexual dimorphic distribution of substance P in specific anterior pituitary cell populations. Proceedings of the National Academy of Sciences USA 88 (4), 1222–1226.

Debeljuk, L., Bandera, R., Bartke, A., 1997. Effect of a non-peptide NK-2 tachykinin receptor antagonist on LH, FSH, and prolactin release by rat hemipituitaries in vitro. Journal of Physiology and Pharmacology 48 (3), 461–478.

Debeljuk, L., Lasaga, M., 1999. Modulation of the hypothalamo-pituitary-gonadal axis and the pineal gland by neurokinin A, neuropeptide K and neuropeptide gamma. Peptides 20 (2), 285–299.

Duval, P., Lenoir, V., Moussaoui, S., Garret, C., Kerdelhue, B., 1996. Substance P and neurokinin A variations throughout the rat estrous cycle; comparison with ovariectomized and male rats: I. Plasma, hypothalamus, anterior and posterior pituitary. Journal of Neuroscience Research 45 (5), 598–609.

Emonds-Alt, X., Vilain, P., Goulaouic, P., Proietto, V., Van Broek, D., Advenier, C., Naline, E., Neliat, G., Le Fur, G., Breliere, J., 1992. A potent and selective non-peptide antagonist of the neurokinin A (NK2) receptor. Life Sciences 50 (15), 101–106.

Grady, E.F., Baluk, P., Bohm, S., Gamp, P.D., Wong, H., Payan, D.G., Ansel, J., Portbury, A.L., Furness, J.B., McDonald, D.M., Bunnet, N.W., 1996. Characterization of antisera specific to NK1, NK2 and NK3 neurokinin receptors and their utilization to localize receptors in the rat gastrointestinal tract. Journal of Neuroscience 16 (21), 6975–6986.

Huang, R., Vicario, P., Strader, C., Fong, T., 1995. Identification of residues involved in ligand binding to the neurokinin-2 receptor. Biochemistry 34 (31), 10048–10055.

Kalra, P., Sahu, A., Bonavera, J., Kalra, S., 1992. Diverse effects of tachykinins on luteinizing hormone release in male rats: mechanism of action. Endocrinology 131 (3), 1195–1201.

Kerdelhué, B., Tartar, A., Lenoir, V., El Abed, A., Hublau, P., Millar, R., 1985. Binding studies of substance P anterior pituitary binding sites: changes in substance P binding sites during rat estrous cycle. Regulatory Peptides 10 (2–3), 133–143.

Larsen, P., Mikkelsen, J., Særmark, T., 1989a. Binding of a iodinated substance P analogue to a NK-1 receptor on isolated cell membranes from rat anterior pituitary. Endocrinology 124 (5), 2548–2557.

Larsen, P., Mikkelsen, J., Mau, S., Særmark, T., 1989b. Binding and internalization of a iodinated substance P analog by cultured anterior pituitary cells. Molecular and Cellular Endocrinology 65 (1–2), 91–101.

Larsen, P.J., Jessop, D.S., Chowdrey, H.S., Mikkelsen, J.D., Lightman, S.L., 1992. Osmotic regulation of substance P and neurokinin A peptide content and substance P binding sites in distinct hypothalamic nuclei of the rat. Peptides 13 (4), 705–712.

Lecci, A., Giuliani, S., Tramontana, M., Carini, F., Maggi, C.A., 2000. Peripheral actions of tachykinins. Neuropeptides 34 (5), 303–313.

Lombet, A., Spedding, M., 1994. Differential effects of non-peptidic tachykinin receptor antagonists on Ca^{2+} channels. European Journal of Pharmacology 267 (1), 113–115.

Lowry, O.H., Rosebrough, A.L., Farr, A.L., Randall, R.J., 1951. Protein measurement with the Folin phenol reagent. The Journal of Biological Chemistry 193, 265–275.

McKee, T., Millar, L., Rodger, I., Metters, K., 1993. Identification of both NK1 and NK2 receptors in guinea-pig airways. British Journal of Pharmacology 110 (2), 693–700.

Nouel, D., Gaudriault, G., Houle, M., Reisine, T., Vincent, J., Mazella, J., Beaudet, A., 1997. Differential internalization of somatostatin in COS-7 cells transfected with SST_1 and SST_2 receptor subtypes: a confocal microscopic study using novel fluorescence somatostatin derivatives. Endocrinology 138 (1), 296–306.

Otsuka, M., Yoshioka, K., 1993. Neurotransmitter functions of mammalian tachykinins. Physiological Reviews 73 (2), 229–308.

Pisera, D., Debeljuk, L., Seilicovich, A., Afione, S., Duvilanski, B., Díaz, M.C., Lasaga, M., Traktenberg, R., Bartke, A., 1991. Possible role of neurokinin A in the physiological control of prolactin secretion in rats and hamsters. Journal of Neuroendocrinology 3, 39–44.

Pisera, D., Duvilansky, B., Lasaga, M., Díaz, M.C., Seilicovich, A., 1994. Stimulation of prolactin release by neurokinin A in vitro. Neuroendocrinology Letters 16, 241–246.

Pisera, D., Theas, S., De Laurentiis, A., Lasaga, M., Duvilansky, B., Seilicovich, A., 1998. The hormonal status modulates the effect of neurokinin A on prolactin secretion in female rats. Journal of Endocrinology 159 (3), 389–395.

Regoli, D., Drapeau, G., Dion, S., D'Orléans-Juste, P., 1989. Receptor for sustance P and related neurokinins. Pharmacology 38 (1), 1–15.

Regoli, D., Boudon, A., Fauchére, J., 1994. Receptors and antagonists for substance P and related peptides. Pharmacological Reviews 46 (4), 551–599.

Renzetti, A., Criscuoli, M., Maggi, C., 1997. Tachykinin NK2 receptors: characterization in the rat small intestine by the use of a peptide agonist and a nonpeptide antagonist as radioligands. Naunyn–Schmiedeberg's Archives of Pharmacology 356 (1), 139–144.

Shamgochian, M., Leeman, S., 1992. Substance P stimulates luteinizing hormone secretion from anterior pituitary cells in culture. Endocrinology 131 (2), 871–875.

Vijayan, E., McCann, S.M., 1979. In vivo and in vitro effects of substance P and neurotensin on gonadotropin and prolactin release. Endocrinology 105 (1), 64–68.