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LOCALIZATION AND FUNCTION OF NK_3 SUBTYPE TACHYKININ RECEPTORS OF LAYER V PYRAMIDAL NEURONS OF THE GUINEA-PIG MEDIAL PREFRONTAL CORTEX

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Abstract—The NK_3 subtype of tachykinin receptor has been implicated as a modulator of synaptic transmission in several brain regions, including the cerebral cortex. The localization and expression of NK_3 receptors within the brain vary from species to species. In addition, the pharmacology of NK_3 receptor-specific antagonists shows significant species variability. Among commonly used animal models, the pharmacology of the guinea-pig NK_3 receptor most closely resembles that of the human NK_3 receptor. Here, we provide anatomical localization studies, receptor binding studies, and studies of the electrophysiological effects of NK_3 receptor ligands of guinea-pig cortex using two commercially available ligands, the NK_3 receptor peptide analog agonist senktide, and the quinolinecarboxamide NK_3 receptor antagonist SB-222,200.

Saturation binding studies with membranes isolated from guinea-pig cerebral cortex showed saturable binding consistent with a single high affinity site. Autoradiographic studies revealed dense specific binding in layers II/III and layer V of the cerebral cortex. For electrophysiological studies, brain slices were prepared from prefrontal cortex of 3- to 14-day-old guinea pigs. Whole cell recordings were made from layer V pyramidal neurons. In current clamp mode with a K^+-containing pipette solution, senktide depolarized the pyramidal neurons and led to repetitive firing of action potentials. In voltage clamp mode with a Cs^+-containing pipette solution, senktide application produced an inward current and a concentration-dependent enhancement of the amplitude and the frequency of spontaneous excitatory postsynaptic potentials. The glutamatergic nature of these events was demonstrated by block by glutamate receptor antagonists. The effects of senktide were blocked by SB-222,200, an NK_3 receptor antagonist.

Taken together, these results are consistent with a functional role for NK_3 receptors located on neurons in the cerebral cortex. In layer V pyramidal neurons of the medial prefrontal cortex, activation of the NK_3 receptor system plays an excitatory role in modulating synaptic transmission.

Key words: neurokinin B, autoradiography, receptor binding, electrophysiology.

The tachykinins are a family of peptides that primarily act as neurotransmitters or neuromodulators in the CNS and peripheral nervous system. The tachykinin family is defined characteristically by the conserved carboxy terminal sequence -Phe-Xaa-Gly-Leu-Met-NH_2, where Xaa is a variable amino acid. The endogenous mammalian tachykinins are substance P (SP), neurokinin A (NKA) and neurokinin B (NKB) (Beaujouan et al., 2004).

These endogenous peptides act preferentially at three tachykinin receptors, termed the NK_1, NK_2 and NK_3 subtype tachykinin receptors. The NK_1 receptor exhibits a higher affinity for the binding of SP than for NKA or NKB (Krause et al., 1994). The NK_2 receptor binds NKA with the highest affinity (Buck and Shatzer, 1998). For the NK_3 receptor, the order of agonist potency is NKB>NKA>SP (Laufer et al., 1986); however, all three ligands are capable of activating the receptor. Like adrenergic, opioid, taste and other G-protein-coupled receptors, the deduced amino acid sequence of the tachykinin receptors is consistent with the seven transmembrane domain structure proposed for this family of receptors.

The pharmacology of tachykinin receptors has been well characterized in pain, and the roles of tachykinin receptor ligands in lacrimation, salivation, and smooth muscle contraction have been well documented (Regoli et al., 1994). Only recently has evidence emerged regarding their role in psychopharmacology. Interest in the role of NK_3 receptors in the brain has been piqued by the development of the brain permeant, potent, NK_3-selective compounds osanetant (Kamali, 2001) and talnetant (Evangelista, 2005).

Studies of the function and pharmacology of NK_3 receptors have proven to be complicated in several respects. One of the difficulties is that the distribution of NKB expressing neurons and of NK_3 receptors in the brain varies among commonly used animal models (Massi et al., 2000; Langlois et al., 2001) and between some of these models and primates (Nagano et al., 2006). The pharmacology of small molecule antagonists also shows interspecies variation. In general, tachykinin receptor antagonists show selectivity for either rat and mouse or guinea-pig and gerbil

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Abbreviations: ACSF, artificial cerebrospinal fluid; AP5, D-(−)-2-amino-5-phosphonopentanoic acid; B_{max}, maximal binding; EPSC, excitatory postsynaptic current; K_d, dissociation constant of the radioligand; K_i, inhibition constant; NBQX, 2,3-dioxo-6-nitro-1,2,3,4-tetrahydrobenzo[f]quinoxaline-7-sulfonamide; NKA, neurokinin A; NKB, neurokinin B; NK_3, NK_3 subtype tachykinin receptor; RT, room temperature; SP, substance P.

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NK_3 receptors (Chung et al., 1995; Emonds-Alt et al., 2002; Sarau et al., 1997, 2000). The pharmacology of these compounds at the human NK_3 receptor is more accurately replicated in guinea-pig and gerbil models than in rat and mouse (Regoli et al., 1994).

Given the relative receptor distribution, pharmacology and potential utility of the guinea pig as an animal model, we have examined the localization and function of NK_3 receptors in the cerebral cortex of the guinea pig. Here, we provide anatomical localization studies, ligand binding studies, and studies of the physiological effects of NK_3 receptor activation and blockade.

EXPERIMENTAL PROCEDURES

Animals

Hartley guinea pigs (Charles River Laboratories, Wilmington, MA, USA) were used in these experiments. Animals were housed in a temperature-controlled vivarium with free access to food and water. Animal experiments were approved by the AstraZeneca IACUC and carried out in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals (NIH Publications No. 80–23). The number of animals used and their suffering were minimized.

Autoradiography

Naïve adult male Hartley guinea pigs were killed by decapitation without anesthesia. Brains were removed and frozen in cooled isopentane (−35 °C). Sections (20 μm) were cut in a cryostat at −15 °C, mounted onto gelatin-coated slides, rapidly dried and stored at −80 °C until use. On the day of the experiment, slides were warmed to room temperature (RT, ~22–25 °C) and preincubated in 50 mM Tris–HCl, 0.005% polyethylamine, pH 7.4. To determine total binding the sections were incubated in the same buffer with 3 mM $MnCl_2$, 0.02% BSA, and 1 nM [^{3}H] SB-222,200 (in house, specific activity 34.7 Ci/mmol) for 60 min. Non-specific binding was determined by adding 10 μM of talnetant (60 min, RT). Slides were rinsed, dried and then exposed to a phosphoimager screen for 5 days. The screens were run on a Cyclone Phosphoimager (PerkinElmer) and images were imported into Photoshop and are unretouched except for the lettering.

Receptor binding

Naïve adult male Hartley guinea pigs were killed by decapitation without anesthesia, the brains were removed and frontal cortical tissue was carefully dissected for membrane preparation. The tissue was homogenized in 20 mM Tris pH 7.4 plus protease inhibitors and centrifuged at 1000×*g* for 5 min. The supernatant was transferred to a new tube and centrifuged at 100,000×*g* for 30 min to pellet cellular membranes. The pellet was resuspended in 20 mM Tris pH 7.4 buffer and protein concentration determined using a standard protein determination kit.

Membranes were resuspended in assay buffer (20 mM Hepes, pH 8.4, 5 mM NaCl, 1 mM $CaCl_2$, 1 mM $MgCl_2$, 0.5 mM $MnCl_2$, 0.1% BSA) containing thiorphan (10 μM), homogenized briefly and resuspended at 0.278 mg/ml for a final concentration of 50 μg/well in 180 μl. For saturation binding experiments, 20 μl [^{125}I]-MePhe^7-NKB was added to triplicate wells at final concentrations ranging from 0.003 nM to 0.75 nM. Non-specific binding was defined by including cold N-MePhe^7-NKB (Bachem, Torrance, CA, USA), osanetant, or senktide (1–10 μM) with the radioligand. The reaction was allowed to equilibrate for 1.5 h at RT and harvested onto GF/B filter plates presoaked in 0.5% BSA. Filters were washed with cold wash buffer (0.02 M Tris–HCl, pH 7, 0.02% BSA), dried and counted on a Topcount instrument (Perkin Elmer, Waltham, MA, USA). For competition binding experiments, compounds were dissolved in DMSO and serially diluted 1:3. Two microliters of each compound concentration was transferred to a 96-well 500 μl polypropylene U-bottom plate for final assay concentrations in the range of 10 μM to 0.17 pM. The [^{125}I]-MePhe^7-NKB ligand was used at concentrations of 0.02–0.07 nM. The remainder of the assay was as described above.

Saturation binding data were analyzed by non-linear regression using GraphPad Prism software to yield K_d (dissociation constant of the radioligand) and B_{max} (maximal binding). For competition binding experiments, inhibition constants (K_i) were calculated using the Cheng-Prusoff equation and XL-Fit software.

Electrophysiology

Coronal slices were prepared from the frontal pole of the brain of guinea-pig neonates of either sex at 2–15 days of age, weighing 40–200 g. Animals were decapitated after induction of deep anesthesia with isoflurane. The brain was rapidly removed and immersed in ice cold artificial cerebrospinal fluid (ACSF) gassed with 95% O_2–5% CO_2. The brain was cut in half with a single coronal cut. The front half of the brain was mounted anterior side up in an ice cold gassed bath for slicing. Slices (200 μm) were cut using a Vibratome (Leica Microsystems). The first four slices were discarded. Subsequent slices were stored in a warm (30 °C) holding chamber gassed with 95% O_2–5% CO_2. Slices were allowed to equilibrate for at least 1 h before recording. Individual slices were transferred to a recording chamber which was perfused with gassed ACSF at 2.0 ml/min. Whole cell recordings of single neurons were conducted at RT, ~23 °C, using an Axopatch 200C (Hamden, CT, USA) patch clamp amplifier. Electrodes with resistances of 4–8 MΩ were filled with pipette solution. Drugs were applied by bath perfusion.

The composition of solutions used for these experiments is listed below, in mM unless stated otherwise. ACSF: 130 NaCl, 3.5 KCl, 1.5 $CaCl_2$, 1.5 $MgSO_4$, 24 $NaHCO_3$, 1.25 NaH_2PO_4, 10 glucose. K-containing pipette solution: 140 K gluconate, 2 $MgSO_4$, 0.1 $CaCl_2$, 0.1 GTP, 2.0 ATP, 10 Hepes, 1 EGTA, pH 7.25. Cs-containing pipette solution: 120 Cs methanesulfonate, 2 $MgCl_2$, 0.3 GTP, 2.0 ATP, 10 Hepes, pH 7.35.

Excitatory postsynaptic currents (EPSCs) were analyzed by the Minanalysis program. EC_{50} values were calculated using the logistic equation: $y=E_{max}\cdot x/(EC_{50}+x)$, where x is drug concentration. Of the neurons tested, 90% responded to senktide. Data were obtained from 52 cells in brain slices from 15 different animals. Data are expressed as the mean±S.E.M.

Chemicals

[^{125}I]-MePhe^7-NKB, specific activity 2200 Ci/mmol, was purchased from PerkinElmer; NKA and SP were purchased from Sigma (St. Louis, MO, USA); senktide, D-(−)-2-amino-5-phosphonopentanoic acid (AP5) and 2,3-dioxo-6-nitro-1,2,3,4-tetrahydrobenzo[f]quinoxaline-7-sulfonamide (NBQX) from Tocris (Ellisville, MO, USA), and N-MePhe^7-NKB from Bachem. SB-222,200 and osanetant were synthesized in house. All other chemicals were purchased from Sigma or Fisher Scientific (Pittsburgh, PA, USA). For the electrophysiological experiments, senktide stock solution at a concentration of 5 mM and SB-222,200 at a concentration of 10 mM were prepared in DMSO. At the highest concentration of senktide tested (2 μM), the concentration of DMSO would be 0.04%. To test for effects of this vehicle, 0.05% DMSO was applied to brain slices. No change in current or sEPSCs was observed (n=4 neurons from two different slices).