Characterization of the Substance P Receptor in Rat Brain Cortex Membranes and the Inhibition of Radioligand Binding by Guanine Nucleotides

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Rat brain cortex membranes bind to a conjugate of substance P and 125I-labeled Bolton-Hunter reagent, and this binding can be inhibited by a low concentration of substance P (Kd = 1.2 ± 0.4 × 10⁻⁸ M). This binding is reversible and saturable (0.5 ± 0.1 pmol of binding sites/mg of protein). Fragments of substance P as small as the carboxyl-terminal hexapeptide can inhibit the binding although their potency decreases with the decrease in the length of the peptides. The binding affinities of smaller peptides or peptides in which the carboxyl-terminal amide or amino acids are removed are drastically reduced. Biologically active analogs of substance P, physalaemin, eledoisin, substance P methyl ester, [D-Ala⁶]hepta(5-11)substance P, kassinin, and the eledoisin-related hexapeptide also can inhibit the binding. However, the binding is not inhibited by polypeptides structurally unrelated to substance P or by amine hormones/neurotransmitters. The binding affinities of biologically active peptides to rat brain cortex membranes are almost identical with their affinities for rat parotid cells which we previously determined. Furthermore, the recently described substance P antagonist, [D-Pro⁵,D-Trp⁷]substance P, inhibits the binding of the 125I-labeled substance P derivative to brain cortex membranes and to parotid cells equally well. These results suggest that the substance P receptors in the brain cortex and the parotid gland are similar. The brain cortex membrane binding of the 125I-labeled substance P derivative can be inhibited by micromolar concentrations of GTP, GDP, and their analogs. ITP and IDP were less active. Adenine and pyridine nucleotides were inactive.

Substance P (Arg-Pro-Lys-Pro-Gln-Gly-Leu-Met-NH₂) is widely located in the central and peripheral nervous systems of mammals (1-4). It displays a variety of pharmalogical actions including stimulation of salivation (5, 6), hypotension (7, 8), vasodilation (8), intestinal contraction (6), hypotension (7, 8), vasodilation (8), intestinal contraction (6), and depolarization of certain neurons (10-12). Substance P has been localized in vesicles close to the synaptic cleft (13-14) and can be released by depolarizing conditions, such as 50 mM potassium, in a calcium-dependent manner both in vivo (15) and in vitro (16, 17). The biosynthesis and transport of substance P has been demonstrated in rat dorsal root ganglia (18, 19) and rat striatum (20, 21). These data strongly suggest that substance P may function as a neurotransmitter/modulator in the central and peripheral nervous systems.

The demonstration of a pharmalogically relevant receptor in nerve tissue is another important criteria for a neurotransmitter. We have previously characterized the binding of substance P conjugated with 125I-BH-SP to the substance P receptor of rat parotid cells (22, 23). We also have shown that the receptor may be a membrane protein (22). Several investigators have noted that the relative potencies of substance P analogs and fragments are different in various pharmacological preparations (22, 24-27). For example, the potencies of substance P fragments to stimulate salivation (6), to bind to the parotid substance P receptor (22), and to lower blood pressure (25) decrease with decreasing peptide length, while in many smooth muscle preparations, substance P(4-11)octapeptide and substance P pyroglutamyl(6-11)hexapeptide are as potent as or more potent than substance P (24, 25). Therefore, it is particularly important to characterize the substance P receptor in different tissues to see if more than one type of receptor exists. In this study, we characterize the binding of 125I-BH-SP to rat brain cortex membranes. Thus, we can directly compare the binding affinities of substance P fragments and analogs to a peripheral receptor (e.g. parotid cells) and to a central receptor.

Since guanine nucleotides (e.g. GTP and GDP) are known to regulate the membrane receptor binding of several polypeptides (28-30) and amine hormones (29-31), we measured the effect of various nucleotides on 125I-BH-SP binding to rat brain cortex membranes. The data show that the brain cortex substance P receptor is specifically regulated by guanine nucleotides.

MATERIALS AND METHODS

Synthetic peptides were purchased from Beckman Instruments (substance P and physalaemin), Peninsula (substance P fragments, [D-Ala⁶]hepta(5-11)substance P, [D-Pro⁵,D-Trp⁷]substance P), or Bachem (substance P free acid, substance P methyl ester, eledoisin). Chymostatin was from Beckman Instruments. Guanylyl-5'-(3'-(R)-methyl)phosphate and guanylyl-3'-(3'- (R)-methylene)diphosphate were purchased from Boehringer Mannheim. All other nucleotides were from Calbiochem-Behring. Monoiodinated 125I-labeled Bolton-Hunter reagent (N-succinimidyl-3-(3'-iodo-4'-hydroxyphenyl)propionate), which was 1500 Ci/mmol at the time of purchase and used within 1 month, was from Amersham Corp. or New England Nuclear.

Preparation of 125I-BH-SP. 125I-BH-SP was prepared as previously described (22, 23), except the radioactive conjugate was purified by reverse-phase liquid chromatography. Monoiodinated 125I-labeled Bolton-Hunter reagent (1 mCi) was flushed with nitrogen to evaporate the solvent. Substance P (6 µg in 20 µl of 0.1 M sodium borate, 1 The abbreviation used is: 125I-BH-SP, substance P conjugated with 125I-labeled Bolton-Hunter reagent.

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pH 8.5, and 1.3 mM acetic acid) was added to the tube and was incubated at 0 °C for 1 h. The reaction mixture was injected onto an Ultrasil-ODS column (Beckman Instruments, 4.6 mm x 25 cm, 5 μm) and eluted with 0.1 M phosphoric acid (buffered to pH 3.5 with triethylamine):acetonitrile (73:27) at 1.4 ml/min at room temperature. Aliquots (5 μl) of each fraction (0.4 min/fraction) were counted, and the radioactive profile was compared to that of a control reaction containing no peptide. Fractions containing monoiodinated 125I-BH-SP were pooled, and mercaptoethanol (50 μM) was added to protect the methionine in the radioactive peptide from being oxidized during storage. Some decomposition appeared to occur during purification, but when stored in the presence of mercaptoethanol, 125I-BH-SP was stable for at least 2 months at -20 °C. The molarity and specific radiopurity.

When stored in the presence of mercaptoethanol, 125I-BH-SP showed two radioactive peaks with retention times of 15.2 and 18.4 min, respectively, which were not present in the reaction mixture containing 125I-labeled Bolton-Hunter reagent without peptide (Fig. 1B). Both of these radioactive products could specifically bind to the brain cortex membrane preparation with high affinity. The quantity of the peak which eluted at 15.2 min varied, and it qualitatively correlated with the amount of the diiodinated reagent present in the monoisodinated 125I-labeled Bolton-Hunter reagent preparation when analyzed by thin layer chromatography. We have not further characterized the binding of this product to the substance P receptor.

The fractions in the major radioactive product peak which eluted at 18.4 min were pooled and used for the receptor binding studies. The separation of 125I-BH-SP from un conjugated substance P is complete so that the specific activity of the radioactive conjugate is the same as that of the unconjugated 125I-labeled Bolton-Hunter reagent (i.e. 1500 Ci/mmol).

125I-BH-SP, purified using reversed-phase chromatography, migrates identically with 125I-BH-SP, purified using thin layer chromatography, on both reversed-phase chromatography and on thin layer chromatography, and it is able to bind to rat parotid cells.

125I-BH-SP could bind to a gradient-purified preparation of rat brain cortex membranes. The time course of this binding is shown in Fig. 2. The binding reached equilibrium after 20 min at 20 °C and was stable for at least 60 min. 125I-BH-SP binding was reduced by 94% when 6.7 μM substance P was included in the incubation. This binding of 125I-BH-SP to avoid oxidation of the COOH-terminal methionine of substance P, it was necessary to recover the 125I-BH-SP from the plate under anaerobic conditions (28). In addition, this method required extreme caution in order to avoid contaminating the work area with radioactive silica particles. For this reason, we have developed a purification procedure using reversed-phase high performance liquid chromatography. The elution profile of such a purification is shown in Fig. 1. 125I-Labeled Bolton-Hunter reagent was incubated in the presence and absence of substance P before injection onto the column. Fig. 1A shows the elution of unconjugated substance P. Chromatography of the reaction mixture containing 125I-labeled Bolton-Hunter reagent and substance P showed two radioactive peaks with retention times of 15.2 and 18.4 min, respectively.

RESULTS

We originally purified the 125I-labeled radioactive derivative of substance P by thin layer chromatography, and in order to...
was discovered that the binding was minimal in the absence of CaCl₂ or MgCl₂ (4-28-fold). MnCl₂ is more effective than CaCl₂ and MgCl₂ with a maximum effect at pH 7.5, containing 50 mM MnCl₂. Aliquots (0.1 ml) were removed, and the membrane-bound radioactivity was determined.

brain cortex membranes in the presence of excess substance P was considered to be nonspecific binding. In subsequent experiments, specific binding is the total amount of 125I-BH-SP bound less the nonspecific binding. The maximum specific binding at 0 and at 37 °C was only 10-20% of that at 20 °C.

Specific binding of 125I-BH-SP to cortex membranes was initially demonstrated using Krebs-Ringer phosphate buffer, which was used previously to characterize the binding of 125I-BH-SP to parotid cells (22, 23). During the course of investigating the effect of various ionic conditions on the binding, it was discovered that the binding was minimal in the absence of divalent cations and the binding was increased by MnCl₂, CaCl₂, or MgCl₂ (4-28-fold). MnCl₂ is more effective than CaCl₂ and MgCl₂ with a maximum effect at 5 mM. In these studies, membranes were incubated with 125I-BH-SP in Krebs-Ringer phosphate and in 0.05 M Tris, pH 7.5, containing various divalent cations. After centrifugation through 10% sucrose, the tip of the microfuge tube was cut and the pellet was extracted with 50 mM MnCl₂. Aliquots (0.1 ml) were removed, and the membrane-bound radioactivity was determined.

We investigated the stability of 125I-BH-SP under the conditions used for the binding assay and also characterized the radioactivity which was bound to the brain cortex membranes. 125I-BH-SP incubated without membranes and analyzed by reverse-phase liquid chromatography, was 80% pure (Fig. 3A). The two minor radioimpurities in the preparation were present even in freshly purified radioactive conjugate, but their level did not increase on storage of the 125I-BH-SP in the presence of 50 nm mercaptoethanol. However, without mercaptoethanol, the impurities comprised greater than 50% of the radioactivity present in the conjugate preparation after only 1 week at storage. Thus, the generation of these impurities appears to involve oxidation of the carboxyl-terminal methionine of the conjugate. The small amounts of these impurities which are seen in Fig. 3 will be readily generated during the extraction and chromatography of 125I-BH-SP.

When 125I-BH-SP was incubated with brain cortex membranes at 20 °C for 20 min, greater than 70% of the unbound radioactivity co-migrated with the major radioactive peak (Fig. 3B). Thus, only 12% of the 125I-BH-SP was degraded under the conditions of the binding assay. We had previously found that the chymotrypsin inhibitor, chymostatin, was useful in preventing the degradation of 125I-BH-SP by parotid cells (22, 23) and this peptide was also required to minimize the degradation of 125I-BH-SP by brain cortex membranes.

However, when 125I-BH-SP was incubated with brain membranes at 37 °C for 30 min in the absence of chymostatin, only 12% of the radioactivity chromatographed with intact 125I-BH-SP. Inclusion of chymostatin (200 pg/ml) in the incubation resulted in the protection of 50% of the radioactive conjugate from degradation. This more extensive degradation of the radioactive label at 37 °C than at 20 °C, even in the presence of chymostatin, may account for the decreased binding observed at 37 °C.

When membrane bound radioactivity was extracted with 50% pyridine, 80% of the radioactivity bound to the membranes was extracted. Reverse-phase chromatography showed that 80% of the radioactivity in the extract was intact 125I-BH-SP (Fig. 3C). Thus, the brain cortex membranes noncovalently bind intact 125I-BH-SP.

Fig. 4A shows that brain cortex membrane binding of 125I-BH-SP was inhibited by low concentrations of substance P. Scatchard analysis of these data (Fig. 4B) is linear, indicating that only one type of binding site is present. The Kᵢ is 12 ± 4 nM and there is 0.5 ± 0.1 pmol of binding site/mg of protein (mean ± S.D., four experiments).

We tested the ability of various fragments and analogs of substance P to inhibit the binding of 125I-BH-SP to brain cortex membranes (Figs. 5 and 6). The binding affinity decreases as amino acids are removed from the NH₂-terminal end of substance P. The substance P pyroglutamyl(6-11)heptapeptide is the smallest fragment which exhibits significant inhibitory activity although, at higher concentrations, substance P(7-11)pentapeptide also inhibits. The biologically active substance P analogs physalaemin, eledoisin, [D-Ala²]-heptapeptide, and substance P in which the COOH-terminal amide was replaced with a methyl ester were all potent inhibitors of 125I-BH-SP binding. The concentrations required to inhibit binding by 50% (IC₅₀) were estimated from these curves and are listed in Table I. Removal of the COOH-
Fig. 4. Competition of brain cortex membrane binding of \(^{125}\)I-BH-SP by substance \(P\) and Scatchard analysis of the binding. \(^{125}\)I-BH-SP (1500 Ci/mmol, \(4.5 \times 10^{-10}\) M) was incubated with brain membranes (0.66 mg/ml) for 30 min at 20 °C as described in the legend to Fig. 2. Various concentrations of unlabeled substance \(P\) were included to compete for the binding of \(^{125}\)I-BH-SP. A, Scatchard analysis of the data in \(B\).

Fig. 5. Displacement of \(^{125}\)I-BH-SP binding to brain cortex membranes by substance \(P\) and its analogs. \(^{125}\)I-BH-SP (750-1500 Ci/mmol, \(4.5 \times 10^{-10}\) M) was incubated with cortex membranes in the absence or presence of competing peptide as described in the legend to Fig. 3. The specific binding in the absence of competitor was taken as 100%.

terminal amide group from substance \(P\) (substance \(P\) free acid) decreases the affinity by 3 orders of magnitude. The NH\(_2\)-terminal (1-9)substance \(P\) fragment has no affinity even at concentrations greater than \(10^{-7}\) M.

A group of substance \(P\) analogs has been shown to antagonize the ability of substance \(P\) to stimulate guinea pig ileum contractions and rat salivation (35) as well as its ability to depolarize certain neurons (36, 37). We have measured the ability of one of these compounds, [D-Pro\(^2\),D-Trp\(^7\)]substance \(P\), to inhibit the binding of \(^{125}\)I-BH-SP to brain cortex membranes and rat parotid cells (Fig. 7). The IC\(_{50}\) of this compound is similar in both systems and is listed in Table I. It is only 0.3% as potent as substance \(P\) in inhibiting \(^{125}\)I-BH-SP binding. This potency correlates well with its reported potency \(in\ v\ i\ t\ o\) and \(in\ v\ i\ t\ u\) (35, 36).

Fig. 8 shows that the potencies of the substance \(P\) fragments and analogs to inhibit \(^{125}\)I-BH-SP binding to brain cortex membranes correlated very well with their potencies to inhibit \(^{125}\)I-BH-SP binding to parotid cells (22) (slope 1.19, correlation coefficient 0.978). This indicates that the binding sites in the two tissues are very similar.

Fig. 6. Displacement of \(^{125}\)I-BH-SP binding to brain cortex membranes by substance \(P\) and its analogs. The incubations were performed as described in the legend to Fig. 5.

Table I

<table>
<thead>
<tr>
<th>Peptide</th>
<th>IC(_{50})</th>
<th>Relative affinity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Substance (P)</td>
<td>1.2 ± 0.5 × 10^{-3}</td>
<td>1</td>
</tr>
<tr>
<td>Substance (P(2-11))decapeptide</td>
<td>2.7 ± 1.3 × 10^{-3}</td>
<td>0.44</td>
</tr>
<tr>
<td>Substance (P(3-11))nonapeptide</td>
<td>5.3 ± 0.3 × 10^{-3}</td>
<td>0.23</td>
</tr>
<tr>
<td>Substance (P(4-11))octapeptide</td>
<td>2.9 ± 1.1 × 10^{-3}</td>
<td>0.04</td>
</tr>
<tr>
<td>Substance (P) pyroGlu(6-11)hexapeptide</td>
<td>2.1 ± 0.1 × 10^{-3}</td>
<td>0.006</td>
</tr>
<tr>
<td>Substance (P(7-11))pentapeptide</td>
<td>4.1 ± 0.9 × 10^{-3}</td>
<td>0.0003</td>
</tr>
<tr>
<td>Substance (P(6-11))tetrapeptide</td>
<td>&gt;2 × 10^{-3}</td>
<td>&lt;0.0006</td>
</tr>
<tr>
<td>Substance (P(9-11))tri peptide</td>
<td>&gt;2 × 10^{-3}</td>
<td>&lt;0.0006</td>
</tr>
<tr>
<td>Substance (P(1-9))nonapeptide</td>
<td>&gt;6 × 10^{-3}</td>
<td>&lt;0.0002</td>
</tr>
<tr>
<td>Substance (P) free acid</td>
<td>1.1 ± 0.5 × 10^{-3}</td>
<td>0.001</td>
</tr>
<tr>
<td>Physalaemin</td>
<td>1.6 ± 0.6 × 10^{-3}</td>
<td>0.75</td>
</tr>
<tr>
<td>Eledoisin</td>
<td>3.8 ± 0.2 × 10^{-3}</td>
<td>0.03</td>
</tr>
<tr>
<td>Substance (P) methyl ester</td>
<td>5.2 ± 4 × 10^{-3}</td>
<td>0.023</td>
</tr>
<tr>
<td>[D-Ala(^3)][6-11]Substance (P)</td>
<td>1.5 ± 0.3 × 10^{-3}</td>
<td>0.08</td>
</tr>
<tr>
<td>[D-Pro(^2),D-Trp(^8)]Substance (P)</td>
<td>3.5 ± 0.03 × 10^{-3}</td>
<td>0.003</td>
</tr>
</tbody>
</table>

Fig. 7. Inhibition of \(^{125}\)I-BH-SP binding to brain cortex membranes and parotid cells by a substance \(P\) antagonist. \(^{125}\)I-BH-SP (1500 Ci/mmol, \(4.5 \times 10^{-10}\) M) was incubated with brain cortex membranes (0.66 mg/ml) as described in the legend to Fig. 5. B. \(^{125}\)I-BH-SP (1306 Ci/mmol, \(4.5 \times 10^{-10}\) M) was incubated with parotid cells (1.7 mg of protein/ml) in the presence and absence of competitor peptide in Krebs-Ringer phosphate, pH 7.0, + 200 μg/ml of bovine serum albumin and 50 μg/ml of chymostatin for 20 min at 20 °C. Bound \(^{125}\)I-BH-SP was separated from the free radioligand by centrifugation through 10% sucrose in a Beckman microfuge.
The ability of 19 different peptides (10^{-5} M) to inhibit 125I-BH-SP binding to brain cortex membranes was determined. Those having no effect on binding were bradykinin, somatostatin, thyrotropin releasing hormone, fMet-Leu-Phe, angiotensin II, neurotensin, ranatensin, parathyroid hormone, tuftsin, bombesin, kemptide, caerulein, glucagon, and adrenocorticotropic hormone. Eledoisin-related peptide and Baclofen, which blocks some central nervous system effects of substance P, but not by irrelevant peptides. We have previously shown that brain cortex membranes contain a saturable, high affinity binding site for 125I-BH-SP. The binding is displaced by biologically active fragments and analogs of substance P, but not by irrelevant peptides. We have previously shown that the relative potencies of 13 substance P fragments and analogs to inhibit 125I-BH-SP binding to parotid cells and to stimulate salivation correlate extremely well (22). We have now shown that the relative potencies of these same fragments to inhibit 125I-BH-SP binding to brain membranes are nearly identical with their relative potencies to inhibit parotid cell binding. This strongly suggests that we are measuring binding to a brain cortex substance P receptor, and furthermore, that this receptor has an identical peptide specificity to the parotid cell receptor.

We determined the ability of several guanine nucleotides to inhibit the binding of 125I-BH-SP (Fig. 9). Both GTP and GDP were very effective inhibitors of binding (IC_{50} = 4.7 and 6.7 μM, respectively). The nonhydrolyzable GTP analog, guanylyl-5’-(β,γ-methylene)diphosphate, was even more potent (IC_{50} = 1.8 μM), while guanylyl-5’-(β,γ-methylene)diphosphate was somewhat less potent (IC_{50} = 33 μM). ATP, cGMP, and cAMP were poor inhibitors. A more complete study of the specificity of this nucleotide effect is shown in Table II. dGTP and dGDP (10^{-4} M) also inhibit the binding of 125I-BH-SP. ITP and IDP (10^{-4} M) were somewhat less effective inhibitors, while CTP and CDP did not inhibit. The monophosphate nucleotides did not inhibit, nor did the nicotine nucleotides.

**DISCUSSION**

We have shown that brain cortex membranes contain a saturable, high affinity binding site for 125I-BH-SP. The binding is displaced by biologically active fragments and analogs of substance P, but not by irrelevant peptides. We have previously shown that the relative potencies of 13 substance

![Fig. 9. Inhibition of 125I-BH-SP binding to brain membranes by nucleotides. 125I-BH-SP (968 Ci/mmol, 4.5 × 10^{-14} M) was incubated with brain membranes for 30 min at 20°C as described in the legend to Fig. 2. The concentrations of nucleotides in the incubation are shown in the abscissa. Gpp(NH)p, guanylyl-5’-(β,γ-imido)diphosphate; Gpp(CH)3p, guanylyl-5’-(β,γ-methylene)disphosphate.](image-url)
Substance P Receptor in Brain Cortex Membranes

neurons. Thus, the substance P fragments containing the carboxyl-terminal hexapeptide and the analogs such as physalaemin and eledoisin can depolarize spinal neurons and also can inhibit the binding of [125I]-BH-SP to rat brain cortex membranes. Smaller fragments have little activity in all three systems. Quantitatively, a major difference was found with substance P (6-11)hexapeptide. This hexapeptide has a binding affinity 0.5% of that of substance P in our binding assay and is 500-1200 and 2.5% as potent as substance P in depolarization of the spinal neurons of rat and frog, respectively (42–44).

Hanley et al. (45) measured the binding of [3H]substance P to rat whole brain membranes, and the relative potencies of substance P fragments and physalaemin to inhibit this binding are similar to what we have reported here. However, proglutamyl(6-11)hexapeptide is slightly more potent (1.7-fold) than substance P in their system, while it is 0.5% as potent as substance P in this report. The discrepancies in the potency of the pyroglutamyl(6-11)hexapeptide among the spinal neuron assays (42-44), the inhibition of binding of [125I]substance P to whole brain membranes (45), and in the receptor assay we report here may reflect differences in receptor populations or may be a result of the greater stability of this NH2-terminal cyclized peptide to proteolytic degradation in crude assay systems. Hanley et al. (45) used crude mitochondrial fraction membranes, whereas we further purified the membranes on a sucrose gradient. We showed that in the presence of chymostatin, even at an elevated temperature (20 °C) which was necessary for maximum receptor binding, only approximately 10% of the [125I]-BH-SP was degraded. We also estimated a higher number of binding sites for cortex membranes (0.5 ± 0.1 pmol/mg of protein) than they determined for whole brain membranes (27.2 fmol/mg of protein) or temporal cortex membranes (4 pmol/mg of protein). At the temperature (0 °C) that they measured the binding, we found that the binding was only 10% of that at 20 °C.

Beaujouan et al. (46) reported specific binding of [125I]BH-SP to a primary culture of mesencephalic cells from mouse embryos. The relative binding affinities of various fragments and analogs of substance P in this system are similar to what we reported here for rat brain cortex membranes and to what we previously reported for isolated rat parotid cells (22, 23). Beaujouan et al. (46) and Hanley et al. (45) found that cells and membranes, respectively, had relatively higher levels of substance P binding sites when obtained from regions of the brain known to contain higher concentrations of substance P.

Nakata et al. (47) and Mayer et al. (48) have measured the binding of [3H]substance P to synaptic membranes of rabbit brain and the binding of an [125I]-Tyr9 analog of substance P to synaptic vesicles of rat brain, respectively. However, this binding did not display the proper peptide specificity, since physalaemin and eledoisin, two biologically active naturally occurring analogs of substance P, did not inhibit binding. Further investigation (48, 49) indicated the binding was to phospholipids of the membranes. Our previous studies have indicated that the substance P receptor on rat parotid cells may be a protein (22).

[125I]-BH-SP has an advantage over [3H]substance P for receptor binding since the former has a higher specific radioactivity. In a comparison study, the specific binding (disaggregations/min) of [3H]substance P (27.6 Ci/mmol, 2.4 × 10−9 M) was only 0.6% that of [125I]-BH-SP (1500 Ci/mmol, 3.4 × 10−10 M).

Although the substance P receptor in brain cortex membranes is similar to that of rat parotid cells, the cellular mediators for substance P may be different in these two tissues. Substance P stimulation of rat parotid tissue slices (50), pancreatic acinar cells (51), and guinea pig ileum (52) does not affect cAMP levels. In the parotid gland, substance P stimulates Ca2+ flux (53) and phosphatidylinositol turnover (54, 55), suggesting that Ca2+ and not cAMP is the mediator of substance P action in this tissue. In contrast, Duffy and Powell (56) and Duffy et al. (57) demonstrated that substance P stimulated adenyl cyclase in rat and human brain, although Quik et al. (58) did not find any increase in cAMP levels in hypothalamic slices incubated with 10 fmol substance P.

It is not possible to measure the effects of guanine nucleotides on [125I]-BH-SP binding to parotid cells since the cell membrane is not readily permeable to nucleotides. Previous studies (28–30) have shown that only membrane preparations and not whole cells are suitable for studies of nucleotide effects. Thus, the question of whether the parotid substance P receptor is regulated by nucleotides can only be answered by measuring the binding of [125I]-BH-SP to a purified parotid membrane receptor. Preliminary experiments with such membranes have indicated that the binding of [125I]-BH-SP to parotid membranes was at least 2 orders of magnitude less sensitive to GTP and GDP than the brain membranes.

The data presented here indicate that the [125I]-BH-SP binding to the brain cortex membrane receptor is modulated by guanine nucleotides. Guanine nucleotide inhibition of hormone and neurotransmitter binding to membrane receptors which are coupled with adenyl cyclase is well documented (29, 30). The inhibition occurs through interaction of the receptor with a nucleotide regulatory protein and with adenyl cyclase (30).

It is not known whether membrane receptors which are not coupled to adenyl cyclase are regulated by guanine nucleotides. It has been proposed that the concept of a regulatory nucleotide subunit may be extended to apply to all hormone-regulated systems (30), but, as yet, there is no evidence that this is the case.

Monovalent and divalent cations have been reported to regulate the affinities of certain hormones for their receptor such as the rat mesenteric artery membrane receptor for angiotensin II (59) and the rat brain membrane receptors for opiates (60, 61). Our assay of the rat brain membrane binding of [125I]-BH-SP depends on centrifugation. The increase of the binding by Mg2+ and Mg2+ can be explained by the increases in the amount of membranes pelleted. However, we could not completely rule out the possibility that these divalent cations may also have a direct effect on the binding, e.g., an increase in the binding affinity or the number of total binding sites. This requires a method which would recover all of the membranes whether or not these divalent cations are present. We have attempted to use filters to separate membranes from the free ligand, but the background values were too high to be useful (see “Materials and Methods”).

REFERENCES

Characterization of the substance P receptor in rat brain cortex membranes and the inhibition of radioligand binding by guanine nucleotides.
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