Regulation of neuropeptide Y (NPY) binding by guanine nucleotides in the rat cerebral cortex

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In the presence of GTP, GDP, GMPP(NH)P, GMPP(CH$_2$)P, GMPP(S)P but not in the presence of GMP, cGMP or ATP, the high affinity binding of neuropeptide Y (NPY) was reduced in a dose-dependent manner. GTP (0.1 mM) diminished the maximal binding capacity for ^{125}I-labelled NPY by 40% without any change in the equilibrium dissociation constant of the receptor ^{125}I-labelled NPY complex.

Neuropeptide Y   Neuropeptide Y-receptor   GTP

1. INTRODUCTION

Neuropeptide Y (NPY) [1,2] is a newly isolated 36 amino acid residues long peptide which occurs in the brain in the highest amounts of all peptides hitherto reported. It is co-stored with noradrenaline in some peripheral neurons [3] and acts synergistically with it at several sites. Recently, a reversible and saturable high affinity binding of ^{125}I-labelled NPY to membranes from rat cerebral cortex, hypothalamus, striatum and hippocampus has been reported [4,5]. We have here examined the effects of guanine nucleotides on the equilibrium binding of ^{125}I-labelled NPY to receptors in membranes from the rat cerebral cortex.

Guanine nucleotides have been shown to be involved in the hormone stimulation and inhibition of the adenylate cyclase in various tissues [6]. Guanine nucleotides lower the binding affinity of agonist in several systems including classical neurotransmitters such as norepinephine [7], acetylcholine [8] and peptides such as substance P [9], somatostatin [10], and cholecystokinin [11]. Activation or inhibition of adenylate cyclase by NPY has not yet been reported. However, guanine nucleotides affect the binding of ^{125}I-labelled NPY causing a decrease in the number of binding sites with virtually no effect on binding affinity of ^{125}I-labelled NPY.

2. MATERIALS AND METHODS

Natural purified porcine NPY was purified by Dr Kazuhiko Tatemoto as in [1] and iodinated by the chloramine-T method of Dr Elvar Theodorsson-Norheim and generously supplied to us.

All chemicals used were of analytical grade. GTP Na$^+$-salt was purchased from Sigma (St. Louis, MO). GMPP(NH)P, GMPP(CH$_2$)P and GMPP(S)P were bought from Boehringer Mannheim.

Adult male Sprague-Dawley rats weighing 150–200 g were decapitated and the brains rapidly removed. Cerebral cortex was dissected, freed from white matter and homogenized in a loose fitting glass-teflon homogenizer in ice-cold sucrose (0.32 M) buffered with 5 mM Hepes (pH 7.4) to yield a 10% (w/v) homogenate.

The homogenate was diluted 10-fold with Hepes-buffered (5 mM, pH 7.4) sucrose (0.32 M) before centrifugation at $1000 \times g$ for 5 min. The resulting supernatant was subjected to further centrifugation at $10\,000 \times g$ for 45 min. The obtained pellet was resuspended in Hepes-buffered (20 mM, pH 7.4) Krebs-Ringer buffer (137 mM NaCl, 2.68 mM KCl, 2.05 mM MgCl$_2$, 1.8 mM CaCl$_2$ and 1 g/l glucose) to yield a protein concentration of 0.25 mg protein/ml. The protein concentration

was determined as in [12] using bovine serum albumin as standard. This crude mitochondrial membrane preparation is referred to as 'membrane' and was used without further fractionation.

Equilibrium binding studies with ^{125}I-labelled NPY were carried out in plastic centrifuge tubes by addition of 0.4 ml of ice-cold protein suspension in Krebs-Ringer buffer to 0.5 ml Hepes-buffered Krebs-Ringer buffer containing BSA (1.0%, w/v) and various amounts of labelled NPY (^{125}I-labelled NPY) and 100 l of nucleotide solutions made up in BSA free Krebs-Ringer buffer. Nonspecific binding was defined as the amount of ^{125}I-labelled NPY bound in the presence of unlabelled NPY (50 pmol/sample).

The incubations were carried out in a waterbath at 37°C for 45 min and were terminated by centrifugation for 1 min in a Beckman Microfuge (at $10\,000 \times g$/min). The supernatant was aspirated off, the pellets were dried and the bottom of the plastic centrifuge tubes containing the pellets were cut off and placed into plastic tubes applicable to the Packard Autogamma 500 D counter equipped with a fixed window for [125]I. The counting efficiency was 61.2%; 8% of the added ^{125}I-labelled NPY was unspecifically bound to the upper part of the centrifuge which was discarded when the bottom containing the pellet was cut off.

3. RESULTS AND DISCUSSION

The equilibrium binding of ^{125}I-labelled NPY

(0-1.5 nM) to receptors in membranes from rat cerebral cortex was decreased by 40% in the presence of GTP (0.1 mM, fig.1A). The Scatchard plot (fig.1B) clearly illustrates that there was no significant change in the K_d value of the binding of ^{125}I-labelled NPY.

The B_{max} values were 444 fmol/mg protein and 265 fmol/mg protein in the absence and presence of GTP (0.1 mM) while the K_d values were 0.32 and 0.28 nM, respectively.

This effect of GTP was dose-dependent as shown in fig.2. The half maximal inhibition of binding of ^{125}I-labelled NPY (0.2 nM) was caused by 10 μM GTP.

Other guanine triphosphate analogs such as GMPP(N)P gave a similar inhibition of ^{125}I-labelled NPY binding (fig.2).

The above nucleotide effects were specific for guanine nucleotides as indicated in table 1. Among the guanine nucleotides GTP and GDP are closely equipotent, while 3'5'cGMP or GMP had no effect. Among the analogs of GTP (GMPP(NH)P, GMPP(S)P and GMPP(CH$_2$)P) GMPP(CH$_2$)P was the least potent as observed in other systems [13] with the imido and sulfur analogs being closely equipotent (cf table 1).

The guanine nucleotide effects observed with most agonists involve conversion of high affinity binding sites into low affinity binding sites with no apparent loss of the total number of receptors labelled by the agonist [7,8]. The present results, however, indicate an apparent loss of receptor sites
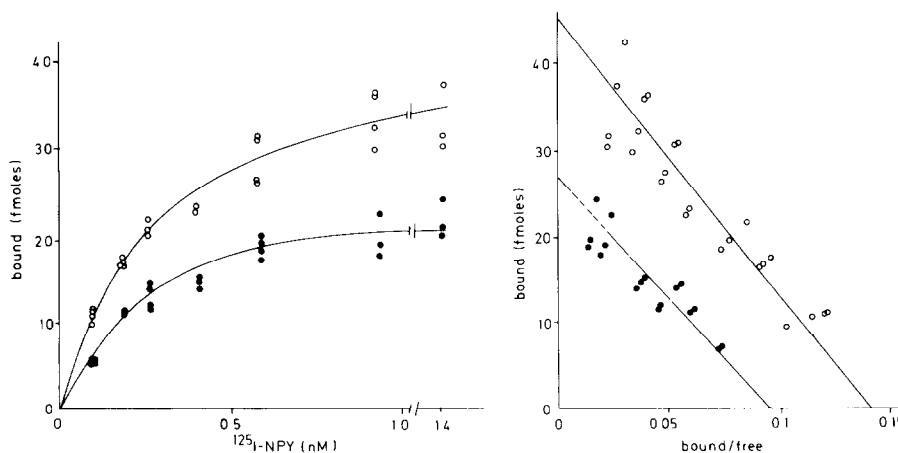

Fig.1. (A) The equilibrium binding of ^{125}I-labelled NPY to membranes from rat cerebral cortex in the absence (○) or presence (●) of GTP (0.1 mM). (B) Scatchard plot of the data in A.
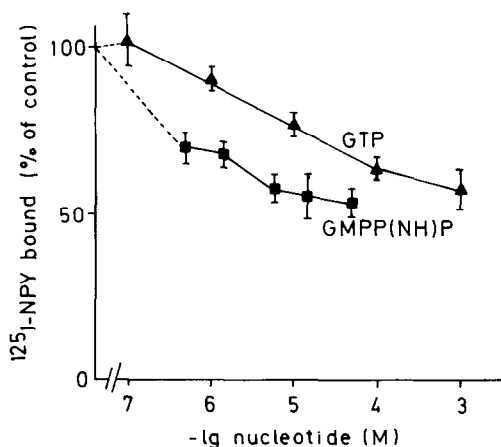
Fig.2. Inhibition of the equilibrium binding of ^{125}I-labelled NPY (0.2 nM) by various concentrations of GTP and GMPP(NH)P.

Table 1

The influence of guanine and adenine nucleotides (0.1 mM) on the equilibrium binding of ^{125}I-labelled NPY (0.2 nM) to receptors in membranes from rat cerebral cortex

Nucleotide (0.1 mM)	% Inhibition of ^{125}I-labelled NPY binding[a]
–	100
GTP	63 ± 3[b]
GDP	69 ± 8[b]
GMP	92 ± 2
3'5'cGMP	96 ± 5
ATP	99 ± 3
AMPP(NH)P	110 ± 4
3'5'cAMP	99 ± 4
GMPP(NH)P	65 ± 5[b]
GMPP(S)P	61 ± 7[b]
GMPP(CH$_2$)P	82 ± 4[c]

[a] Mean ± SD of quadruplicate determinations. 100% ^{125}I-labelled NPY binding corresponds to 444 fmol/mg protein
[b] Significantly different from control, $p < 0.05$
[c] Significantly different from control, $p < 0.1$

for the agonist. To our knowledge there are two peptide hormone systems where similar results were obtained: (a) in the presence of GTP or GMPP(NH)P, almost 90% of the [^{125}I]iodo-N-Tyr-somatostatin binding disappears without a change in the binding affinity for the remaining

sites [10]; (b) in the presence of GMPP(NH)P, 60% of substance P binding sites in membranes of rat salivary gland disappear without any change in the affinity for the remaining sites [9]. In addition, binding of the β-agonist β-hydroxybenziliso-proterenol, shows both GTP-effects; reduction in the number of binding sites and change in affinity of agonist [14].

Plausible explanations for these effects are: (a) a GTP-caused desensitization; (b) strong negative cooperativity between agonist binding sites, induced by a heterologous interaction with GTP; (c) the presence of two subpopulations of NPY receptors of which one is not coupled to any GTP binding protein and therefore unaffected by GTP binding, and another subpopulation which is coupled to GTP binding proteins and suffers such a large loss of affinity in the presence of GTP that NPY binding to it using the limited concentration range of ^{125}I-labelled NPY (fig.1) could not be detected.

Lack of structural data on NPY receptors prevents distinction between the above alternatives for the time being.

Guanine nucleotide-caused changes in the equilibrium binding of agonists are usually interpreted as indicating that the agonist can either activate or inhibit the adenylate cyclase via a guanine nucleotide binding protein [6]. In the case of neuropeptide Y, we have so far not been able to demonstrate stimulation of cyclic AMP synthesis by NPY or inhibition of β-agonists-induced cyclic AMP synthesis by NPY in tissue blocks from the rat cerebral cortex.

Nevertheless, the experiments above suggest that a guanine nucleotide binding protein (activatory or inhibitory) in the adenylate cyclase system [6] can be coupled to the NPY receptors and is capable of extensively influencing the binding of ^{125}I-NPY.

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REFERENCES

[1] Tatemoto, K., Carlquist, M. and Mutt, V. (1982) Nature 296, 659–660.
[2] Tatemoto, K. (1982) Proc. Natl. Acad. Sci. USA 79, 5485–5489.

[3] Emson, Y.C. and De Quidt, M.E. (1984) Trends Neurosci. 7, 31–35.

[4] Undén, A., Tatemoto, K. and Bartfai, T. (1983) Abstracts Society for Neuroscience, part 1, 170.

[5] Undén, A., Tatemoto, K., Mutt, V. and Bartfai, T. (1984) submitted.

[6] Rodbell, M. (1980) Nature 284, 317–322.

[7] Kent, R.S., DeLean, A. and Lefkowitz, R.J. (1980) Mol. Pharmacol. 17, 14.

[8] Berrie, C.P., Birdsall, N.J.M., Burgen, A.S.V. and Hulme, E.C. (1979) Biochem. Biophys. Res. Commun. 87, 1000.

[9] Lee, C.M., Javitch, J.A. and Snyder, S.H. (1983) Mol. Pharmacol. 23, 563–569.

[10] Enjalbert, A., Rasolonjanahary, R., Moyse, E., Kordon, C. and Epelbaum, J. (1983) Endocrinology 113, 822–824.

[11] Innis, R.B. and Snyder, S.H. (1980) Eur. J. Pharm. 65, 123–124.

[12] Peterson, G.L. (1977) Analyt. Biochem. 83, 346–356.

[13] Cascieri, M.A. and Liang, T. (1983) J. Biol. Chem. 258, 5158–5864.

[14] Williams, L.T. and Lefkowitz, R.J. (1977) J. Biol. Chem. 252, 7207–7213.