Natriuretic Peptide Receptors in Cultured Rat Diencephalon*

(Received for publication, December 11, 1989)

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To characterize the type of cell expressing natriuretic peptide receptors in the brain and the nature of these receptors, we conducted studies in primary cultured glial and neuronal cells derived from fetal rat diencephalon. The glial predominant cultures (95% of total cells and glial fibrillary acidic protein positive) expressed nearly a 10-fold greater specific binding of the natriuretic peptides to cell surface receptors compared with the neuron-predominant cultures. Scatchard analysis of binding studies with [125I]-atrial natriuretic peptide (ANP) and [125I]-brain natriuretic peptide (BNP) revealed a single class of receptors with dissimilar affinities (0.25 ± 0.09 and 0.74 ± 0.07 nM, respectively, n = 3 experiments p < 0.01) but similar numbers of binding sites for both peptides (93 and 88 fmol/mg of protein, respectively). Cross-linking of [125I]-ANP and BNP to cultured glia followed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis and autoradiography identified distinct bands at either approximate M, 130,000, or 102,000 and 66,000, corresponding to two high molecular weight (B) receptors and one low molecular weight (C) receptor described in other tissues. Different subtypes of astrocytes appeared to express different B receptors. Binding and cross-linking of radiolabeled ANP or BNP were competitively inhibited equally by unlabeled ANP or BNP, indicating that ANP and BNP probably bind the same receptors. The glial cultures functionally expressed a receptor(s) with guanylate cyclase activity; BNP was less potent than ANP in stimulating cGMP at lower concentrations. These results indicate that both high and low molecular weight natriuretic peptide receptors are expressed in astrocyte-predominant cultures from the fetal diencephalon and suggest that glia participate in several actions of ANP which are probably mediated through this area of the brain.

ANP and BNP are the two most abundant members of a family of polypeptides synthesized in several organs in the body (1–3). In the brain, ANP and BNP and natriuretic peptide receptors have been localized in discrete yet widespread areas (4–6). BNP is present in much greater concentrations than ANP in the porcine brain (6), and the distribution of the peptides suggests important roles for ANP and BNP as neuromodulators of blood pressure (7), cerebrospinal fluid production (8), and hormone secretion and action (9–11).

Two classes of natriuretic peptide receptors have been characterized in peripheral tissues. A “B” (or biologically active) receptor with approximate molecular weight of 135,000 has been found in many tissues including kidney, adrenal, and endothelium (12–14). Binding of the natriuretic peptides to this receptor results in the generation of cGMP and therefore mediates many of the actions of ANP and BNP. A comparably large molecular weight protein from rat brain has been isolated, cloned, and expressed in COS-7 cells, revealing intrinsic guanylate cyclase and ANP-binding activity (15) and may account for the in vitro findings that ANP can induce production of cGMP by the choroid plexus (8, 16). Recently, another B receptor of approximate M, of 115,000 has been cloned from a human placental cDNA library, expressed in various tissues including rat brain, and has also been shown to contain intrinsic guanylate cyclase activity (17). A second receptor class of lower molecular weight (66,000) and lacking guanylate cyclase activity has been described in several peripheral tissues (18, 19). This “C” receptor apparently functions as a clearance receptor (20). It has not been established that this receptor is present in the brain.

In this work glial and neuronal cultures from fetal rat diencephalon, an area known to contain high concentrations of natriuretic peptides and undetermined types of ANP receptors in adult animals (4, 21), were used to examine what type of cell expresses receptors for these hormones. We also determined whether both B (guanylate cyclase containing) and C receptors were present and compared the ability of the various natriuretic peptides to generate cGMP, indicating a functional group of receptors. Finally, we determined whether ANP and BNP act at the same receptors in the brain and compared their relative affinities and binding characteristics.

EXPERIMENTAL PROCEDURES

Materials—Rat ANP and BNP were obtained from Peninsula Labs (Belmont, CA), and [125I]Nal was obtained from ICN. ANP 423 was a generous gift from Dr. Robert Scarborough of California Biotechnology. Antibodies to cGMP and [125I]-cGMP were obtained from Chemicon Corp. and ICN, respectively. Bis-sulfosuccinimidyl was obtained from Pierce Chemical Co. Papsin, neutral protease, and DNase were obtained from Worthington and Boehringer Mannheim, respectively. DMEM/F-12, Fungizone, and fetal bovine serum were from Irvine Scientific, and 5-FUDR was from Aldrich. HBSS was from Gibco and poly-D-Lysine from Sigma. Antibodies to neuron-specific endolase and GFA were obtained from ICN and Polysciences, respectively. Anti-mouse and anti-rabbit antibodies were from Chemicon and Dakopatts.

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Cell Cultures—The diencephalic area of the brain was carefully isolated from 16-day gestational fetal rats and placed into sterile HBSS medium. The tissue consisted mainly of hypothalamus with some thalamus (estimated to be 15% of total tissue) attached. The cells were then mechanically and enzymatically dispersed (92). Briefly, the HBSS was aspirated, the tissue washed three times with 5 ml of HBSS at room temperature, and 5 ml of PPD (0.01% papain, 0.1% neutral protease, 0.01% DNase) was added to the tube, incubated for 15 min at 37 °C, and then triturated five times with fire-polished Pasteur pipettes. The tubes were incubated at 37 °C for an additional 30 min. The tissue was then triturated 10 times, incubated for 30 min, and triturated an additional 5-10 times, then centrifuged at 1500 X g for 5 min. The PPD was then removed and the cell suspension was incubated for 15 min at 37 °C, triturated several times, and centrifuged (reducing the DNase). The suspension was then incubated for 15 min, triturated twice as described, centrifuged, and the DMEM/F-12-DNase solution aspirated. The cell pellets were resuspended in 10 ml of complete DMEM/F-12 medium (1:1) with 10% fetal bovine serum, 200 units/ml penicillin, 200 μg/ml streptomycin, and 500 ng/ml P选gonzone and 15 mM HEPES) and passed through an 80-μm Nitex screen into a conical tube and diluted in a total of 30-40 ml. A 0.5-ml cell sample was removed for cell counting and trypan blue exclusion. The cells were then plated at an optimal density of 5 X 10^5 cells/cm² in DMEM/F-12 medium with 10% fetal bovine serum and kept in a 37 °C incubator in 5% CO₂ air and 1% H₂O₂. Medium was changed every 3rd day, and 5-FUDR (100 μM) was added on day 3 after plating, to decrease glial cell overgrowth. For binding and cGMP generation studies, we omitted the addition of 5-FUDR to allow the glia to proliferate and overgrow the cultures. In some cases, trypsin detachment and reattachment were used to promote glial predominance. The tissue was maintained up to 30 days at -70 °C using Cronex intensifying screens. Laser densitometric analysis (LKB) was used to compare the intensity of autoradiographic bands. Autoradiographic band density was determined using the migration of known protein standards (Bio-Rad).

Immunocytochemistry—Representative cultures were stained to determine the relative proportions of neurons and glia. Glia were labeled by staining with a monoclonal antibody to glial fibrillary acidic protein (GFAP) at a dilution of 1:500. Neurons were labeled with antibodies raised against neuron-specific enolase at a dilution of 1:400. Cultures were first fixed in 4% paraformaldehyde in phosphate-buffered saline for 30 min at room temperature, then in acid alcohol (1% acetic acid, 95% ethanol) for 5 min. Following a 10-minute wash with PBS, cultures were incubated in blocking buffer (PBS with 20% normal goat serum for 30 min. Primary antibodies were then applied at appropriate dilution in PBS with normal goat serum at 37 °C for 24 h in a humid environment. Cultures were washed four times, a total of 30 min in PBS and incubated with appropriate secondary antibodies conjugated to fluorescein isothiocyanate for 1 h at room temperature. For GFAP, the secondary was anti-mouse at 1:100 and for neuron-specific enolase, anti-rabbit at 1:500. Cultures were then washed with PBS for 30 min and coverslipped with 10% glycerol in PBS. Analysis was carried out with an Olympus IMT-2 microscope with epifluorescence illumination.

Binding Studies—Varying concentrations of 125I-ANP and BNP (0.05-2 nM) were added to triplicate wells of cultured neurons or glia and incubated for 90 min at 4 °C to equilibrium, based upon our time course binding studies. The iodinated ligands were prepared by an IOD-OGEN method (23) to a specific activity of 200-450 Ci/mmol. The labeled peptides were added to a specific activity of 200 μCi/μg for these experiments. Non-specific binding was determined by adding 10^7 M unlabeled peptide and always accounted for less than 10% of total binding. Acid washing eliminated over 90% of specifically bound counts. Competition binding studies were carried out by adding 0.1 nM 125I-ANP or BNP to a series of unlabeled ligand concentrations. The labeled peptide in the culture medium were greater than 90% undegraded after a 90-min exposure to glia, as assessed by high pressure liquid chromatography.

Cross-linking Procedure—Glia cells were labeled by adding 1 X 10^6 cpm of 125I-ANP or BNP in our usual binding buffer containing protease inhibitors at 4 °C for 90 min. The receptor-ligand complex was then centrifuged, the pellet washed three times in sodium phosphate buffer, and then cross-linked with 0.5 mM bis-sulfosuccinimidyl carbonate in 2 ml of PBS and incubated for 60 min at 4 °C. The cross-linking solution was removed from the cells by aspiration followed by the addition of 2 ml of cold 50 mM Tris-Cl (pH 7.4) in PBS for 15 min. The cells were then scraped and pelleted by centrifugation at 2500 X g for 10 min and the supernatant was removed and replaced with PBS and then with cold Tris-Cl. Specific binding and cross-linking of labeled ANP and BNP were carried out in the presence of 0.1 μM "cold" ANP 1-28, ANP 4-23, or BNP. The obtained pellet was resuspended in 50 μl of SDS-solubilization buffer (10 mM Tris-HCl (pH 6.8), 1% SDS, 27% glycerol, in the presence or absence of 50 mM dithiothreitol (reducing) and 0.05% bromphenol blue) and heated to 95 °C for 5 minutes.

After cooling, the samples (about 0.3 mg of protein, by Lowry) were analyzed by one-dimensional SDS-polyacrylamide gel electrophoresis on a 20-75% separating gel for 5 h at 200 volts, according to the method of Laemmli (24). Following electrophoresis, the gels were stained overnight in 40% methanol, 10% acetic acid, and 0.1% Coomassie Brilliant Blue R, destained in 40% methanol and 10% acetic acid, and dried for 2 h at 80 °C under vacuum. Autoradiograms were generated by exposing the dried gels to Kodak XAR-5 film for up to 30 days at -70 °C using Cronex intensifying screens. Laser densitometric analysis (LKB) was used to compare the intensity of autoradiographic bands. Autoradiographic band density was determined using the migration of known protein standards (Bio-Rad).

Guanosine Cyclic 3',5'-Monophosphate Generation—To examine which cells are responsible for the generation of cGMP in response to the natriuretic peptides and the magnitude and potential differences in generating cGMP, the following studies were carried out. Glial and neuronal cultures were washed and incubated for 15 min at 37 °C in culture media without fetal bovine serum. Isobutylmethylxanthine (0.5 mM) was then added to all wells for 5 min, followed by the addition in triplicate wells of buffer (control), ANP 1-28, BNP, ANP 4-23, and ANP 5-28 (the major form of ANP secreted from our cultured neurons (25), in concentrations ranging from 1 to 100 nM, for an additional 5 min. The reaction was then stopped by the addition of 1 N HCl, the walls scraped, and the supernatants lyophilized after centrifugation. The samples were reconstituted in radioimmunoassay buffer (0.1 M sodium acetate, pH 5.8) after the addition of NaOH to neutralize the HCl. The selection of a 5-min incubation was based upon time course studies conducted in these cells. The radioimmunounassay for cGMP was a double antibody separation, equilibrium assay. Sensitivity of the assay was 50 fmol/100 μl in nonacetylated samples, and the inter- and intra-assay variations were less than 10%.

Data Analysis—Binding studies were transformed and analyzed by Scatchard analysis using the Ligand program (D. Rodbard, NIH), which generated dissociation constants and receptor densities. These data were then compared by an unpaired Student's t test. A significant F test was then analyzed further by Scheffe's test. A p value < 0.05 was considered significant. All studies were repeated at least three times.

RESULTS

Immunocytochemistry—In glial predominant cultures, approximately 95% of cells were GFAP immunoreactive. Morphologically, many cells exhibited processes indicating that they were glia, as shown by their immunopositivity for glial, epithelial, and probably fibroblasts constituting the remainder of the cells.

Binding Studies—Between 18 and 27% of radiolabeled ANP and BNP specifically bound to the glial cultures in eight separate experiments, whereas binding to the neuronal cultures was only 2-3%. The small amount of binding in the neuronal cultures was probably attributable to the 5-10% of cells that were glia, as shown by their immunopositivity for GFAP. Binding equilibrium was reached at 90 min for both...

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labeled peptides, and nonspecific binding was less than 10% of total binding. The specificity of binding was established further since insulin, somatostatin, and β-endorphin did not displace labeled ANP, even at 10^{-7} M (data not shown). In the glial cultures, Scatchard analysis of the binding data suggested a one-site receptor model with a $K_D$ for ANP and BNP of 0.25 ± 0.09 and 0.74 ± 0.07 nM ($\mu < 0.01$, $n = 3$ experiments), whereas receptor density was 93.2 ± 0.5 and 88 ± 0.3 fmol/mg of protein, respectively (Fig. 1, inset). Competitive inhibition studies of radiolabeled ANP and unlabeled ANP 1-28, ANP 4-23, and BNP revealed different inhibitory characteristics for the three ligands with IC$_{50}$ values of 0.23, 0.43, and 0.64 nM, respectively (Fig. 2); two additional studies yielded similar results. These data suggest that BNP is less potent than ANP at displacing $^{125}$I-ANP from the natriuretic receptor binding sites in brain and is consistent with the decreased affinity shown by Scatchard analysis. The results also suggest that ANP 4-23, a C receptor ligand, is interacting at only a proportion of the receptors since fewer counts were displaced (higher residual counts remained) at all concentrations. This hypothesis was confirmed in the cross-linking studies, which showed that binding and cross-linking of labeled peptide to the receptors were never as well competed off by ANP 4-23 as by ANP 1-28 or BNP even at a concentration of 0.1 μM (see below). Nevertheless, the small differences in displacement compared with ANP 1-28 or BNP suggested that ANP 4-23 binds to the vast majority of receptors in glia, a concept supported by studies in peripheral tissues (13).

**Affinity Cross-labeling of Natriuretic Peptide Binding Sites—**Cross-linking of $^{125}$I-ANP and BNP in glial cultures clearly identified several prominent bands. Interestingly, some glial preparations showed only M, 102,000 and 66,000 bands (Fig. 3, lanes 1 and 3), whereas in other cultures only the 130,000 and 66,000 M, bands were prominently expressed (Fig. 4). Our observations indicated that the 102,000-dalton receptor was expressed in cultures that morphologically resembled type 1 astroglia, whereas the 130,000 M, receptor was seen in cultures that morphologically were predominantly type 2 astrocytes; all glial cultures expressed a 66,000 M, receptor. In the presence of 100 nM ANP 1-28, the circulating form of the peptide in plasma which binds both B and C receptors, labeling of all bands by both $^{125}$I-ANP and BNP was strongly and equally inhibited. Furthermore, unlabeled BNP at this same concentration competed off binding by $^{125}$I-ANP or BNP comparably to unlabeled ANP 1-28. Unlabeled ANP 4-23 competed off equally the binding of both $^{125}$I-ANP or BNP only at the lower molecular weight band, presumably the C receptor. By densitometry, greater than 95% of labeled peptide cross-linked to this band. Since the affinity of the B and C receptors is the same by Scatchard analysis, these results indicate the predominance of this class of receptor in glia. When $^{125}$I-ANP was cross linked and run under reducing or nonreducing conditions (with and without dithiothreitol), both high and low molecular weight bands were identified (Fig. 4, lanes 3 and 5). This indicated that the M, 130,000 band was not simply an aggregate of the 66,000 molecular weight receptor.

**cGMP Generation—**The generation of cGMP in our glial system was dose dependent and similar for ANP 1-28 and ANP 5-28, the most abundant form of ANP in our neuronal cells (25) (Fig. 5). In contrast, BNP-induced cGMP generation was significantly less than ANP at all concentrations except 10^{-6} M. No cGMP was generated above basal levels by ANP.
43,000; bovine serum albumin, 66,000; phosphorylase plus 0.1

carried out as described under "Experimental Procedures," and the

uretic peptides and receptors in the adult (4, 21), specific

5-8, "B-NP alone, labeled BNP plus 0.1

pM

respectively. Molecular weight markers (Bio-Rad) are ovalbumin,

ANP, and labeled ANP plus 0.1

7.5'S gels were subjected to autoradiographic analyses.

cells, an area known to contain high concentrations of natri- 

4-23 at lo-' M in the glial cultures; this indicated the specific-

ity of this ligand for the non-guanylate cyclase-linked C

receptor, which is not primarily responsible for the generation

of this second messenger. ANP 4-23 also did not affect the

generation of cGMP in neuron-predominant cultures was

less than 5% of that in the glial predominant cultures.

DISCUSSION

Our findings indicate that in cultured fetal rat diencephalic

cells, an area known to contain high concentrations of natri-

uretic peptides and receptors in the adult (4, 21), specific

natriuretic peptide receptors are present in cultures that are

95% glia. In contrast, neuron-predominant cultures showed

nearly 10-fold less binding, strongly implicating astrocytes as

the cell-expressing natriuretic peptide receptors. These results

must be extrapolated cautiously to the in vivo brain since

culture conditions may alter any expression of receptors on

neurons. Affinity cross-linking studies clearly showed that

the glial predominant cultures express both B (guanylate
cyclase-linked) and C receptors and exhibit binding at these

receptors by both of the major natriuretic peptide groups in

the brain. This demonstrates that both ANP and BNP bind

the same receptors. The vast majority of receptors expressed

are low molecular weight (probably C receptors). Both BNP

and ANP induce the generation of cGMP, which is consistent

with the existence of a functional B receptor population in

our cultured glia. Interestingly, a particular glial culture ex-

pressed one type of B receptor and the C receptor but never

both B receptors. Our observations indicate that morphologic

subtypes of astrogia express different B receptors, but pre-

cise determinations await homogeneous cultures of type 1 and

type 2 astrocytes.

Previous studies in the adult rat brain have used immuno-

histochemistry and autoradiography to identify ANP binding

sites (5), but these studies did not identify the type of cell

demonstrating binding of labeled peptide. Studies using mem-

brane fractions from whole choroid plexus indicated that

the epithelial cells bind ANP and generate cGMP in response to

the peptide, perhaps mediating cerebrospinal fluid production

(8). Simonnet et al. (26) have identified ANP receptors on

cultured mouse spinal cord glia. In contrast, single cell re-

cordings of the action of injected ANP on neurons in the

nucleus of the solitary tract showed that ANP-induced exci-

tation of the firing rate correlated with a physiologic function

of these neurons (27), presumably mediated by specific ANP

FIG. 4. Autoradiograms of SDS-polyacrylamide gel electro-

phoresis of 125I-ANP and 125I-BNP (2 nM) bound and cross-

linked to primary cultures of predominantly type 1 astroglial

cells (1.1 mg of glial protein). Binding and cross-linking were

carried out as described under "Experimental Procedures," and the

7.5% gels were subjected to autoradiographic analyses. Lanes 1-4,

125I-ANP, labeled ANP plus 0.1 uM ANP 1-28, labeled ANP plus 0.1

uM ANP 4-23, and labeled ANP plus 0.1 uM BNP, respectively. Lanes

5-8, 125I-BNP alone, labeled BNP plus 0.1 uM BNP, labeled BNP

plus 0.1 uM ANP 1-28, and labeled BNP plus 0.1 uM ANP 4-23,

respectively. Molecular weight markers (Bio-Rad) are ovalbumin,

43,000; bovine serum albumin, 66,000; phosphorylase b, 97,000; 

ß-galactosidase, 116,000; myosin, 200,000.

FIG. 5. Generation of cGMP in fetal rat diencephalic glia

cell cultures induced by varying concentrations of natriuretic

peptides compared with basal (absence of peptides) cGMP

levels (B). Glia containing wells were washed free of serum and

incubated at 37 °C in DMEM/F-12 medium for 15 min, then prein-

cubated with 0.5 mM isobutylmethylxanthine for 5 min, followed by

incubation with medium (control) or various concentrations of natri-

uretic peptides in triplicate. The reaction was stopped by the addition

of 0.1 N HCl, and cGMP was determined by radioimmunoassay. Each

bar represents the mean ± S.E. of triplicate determinations per

experiment from the combined results of two experiments, represent-

ative of a third study. ANP 1-28 (O), ANP 5-28 (W), BNP (0) and C-

ANP 4-23 (X) are depicted. *p < 0.01 by analysis of variance and

multiple range tests for basal versus cGMP at a specific concen-

tration. ** p < 0.01 for BNP compared with ANP.

4-23 at 10^-7 M in the glial cultures; this indicated the specific-

ity of this ligand for the non-guanylate cyclase-linked C

receptor, which is not primarily responsible for the generation

of this second messenger. ANP 4-23 also did not affect the

ability of ANP 1-28 to generate cGMP (data not shown).

Generation of cGMP in neuron-predominant cultures was

less than 5% of that in the glial predominant cultures.

Our findings indicate that in cultured fetal rat diencephalic

cells, an area known to contain high concentrations of natri-

uretic peptides and receptors in the adult (4, 21), specific

ANF and BNP Receptors in the Brain

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receptors. Preliminary results from Beaumont and Tan have identified natriuretic peptide receptors on cerebrocortical astrocytes, and in recent preliminary studies, we have found a comparable degree of binding of ANP and BNP to cultured glia prepared from the fetal rat olfactory bulb. Together, these studies indicate the diversity of cells that express natriuretic peptide receptors, although in several areas of the brain it appears that receptors are found mainly on glia. Therefore, the type of cell expressing natriuretic peptide receptors might provide insight as to the function of ANP or BNP in a particular brain area or nucleus. ANP has been postulated to act as a neurotransmitter, regulating such diverse functions as fluid and electrolyte balance, blood pressure, and hormone secretion and action in the brain (9–11). Our diencephalic cultures consist mainly of hypothalamic cells, an area of the brain which in vivo probably participates in several of the functions mentioned. ANP could be playing a paracrine role, regulating several important physiologic processes through glial cells. As an example, messenger RNA for angiotensin has been detected in glia, and ANP has been found to oppose the action and probably the production of angiotensin II in the peripheral circulation (29). Thus, the regulation of angiotensin production/secretion from glia may be an important level at which ANP limits the effects of angiotensin in the brain (30). Alternatively, glial growth or maturational factors (31, 32) could be affected by the natriuretic peptides. In this way, ANP could influence both glial and neuronal function and maturation and might limit the production/secretion of natriuretic peptides from the neurons in feedback fashion.

The distribution and binding of BNP to receptors in the brain have not been described. Peptide mapping studies have demonstrated widespread yet discrete localization of BNP in the brain in concentrations as much as 10-fold higher than ANP (6). Our studies indicate that BNP receptors in cultured diencephalic glia are probably the same as ANP receptors, although BNP binds to these receptors with a lower affinity and generates less cGMP. Since porcine BNP was used in these studies, the different affinities might reflect species differences in the ligand since, unlike ANP, the structure of BNP varies markedly between rat and pig (33). However, similar affinities for porcine BNP and rat ANP have been demonstrated in cultured rat aortic smooth muscle and endothelial cells (34). Our findings are consistent with the different affinities of porcine BNP and rat ANP for natriuretic peptide receptors in rat peripheral sympathetic ganglia (35). These results also extend the findings that BNP binds less avidly than ANP in COS cells transfected with the cDNA of either of the two B receptors (15).

The binding and cross-linking studies utilizing ANP 4-23 provide compelling evidence that the vast majority of ANP receptors in cultured fetal rat diencephalon are C receptors. ANP 4-23 competed off greater than 95% of the binding of labeled ANP and BNP, indicating the predominance of the C receptor in our cell population. These studies also indirectly identified the presence of B receptors since the residual binding to a protein of M, 130,000 or 102,000 designates these bands as the B receptors. Only the 115,000 dalton B receptor cDNA has been cloned from human brain, whereas both B receptor cDNAs have been found in rat brain (15). Our glial

**REFERENCES**


ANF and BNP Receptors in the Brain

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