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DOPAMINE RELEASE FROM NERVE ENDINGS INDUCED BY POLYSIALOGANGLIOSIDES

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SUMMARY: Polysialogangliosides but not monosialoganglioside or a neutral glycosphingolipid induce release of [^{3}H]-dopamine from synaptosomes in presence of Ca^{++}, presumably by exocytosis. This effect is discussed in relation to the ability of polysialogangliosides to induce membrane fusion in chicken erythrocytes and to their behaviour in lipid monolayers. It is suggested that characteristic interactions with phosphatidylcholine involving decreases of surface potential are participating in the polysialoganglioside-induced neurotransmitter release.

The release of neurotransmitter occurs by different processes including calcium-dependent exocytosis in which the synaptic vesicle fuses with the synaptosomal plasma membrane (1). The process of membrane fusion can be induced "in vitro" in the presence of Ca^{++} by polysialogangliosides but not by monosialogangliosides or neutral glycosphingolipids in an experimental system employing chicken erythrocytes (2). This property and the fact that polysialogangliosides are normal constituents of nerve endings suggest that they may participate in the events leading to neurotransmitter release. As a first approach to the investigation of this possibility we studied the influence that different gangliosides added exogenously may have on the release of dopamine from synaptosomes previously loaded with this neurotransmitter.

MATERIALS AND METHODS

Synaptosomal loading and release of dopamine. Female rats (200-250 g body weight) were injected intraperitoneally with pheniprazine

Abbreviations: Gg_4Cer, Galβ1→3GalNAcβ1→4Galβ1→4Glcβ1→1Cer; GD_3, NeuAcα2→8NeuAcα2→3Galβ1→4Glcβ1→1Cer; GM_1, Galβ1→3GalNAcβ1→4Gal(3←2αNeuAc)β1→4Glcβ1→1Cer; GD_{1a}, NeuAcα2→3Galβ1→3GalNAcβ1→4Gal(3←2αNeuAc)β1→4Glcβ1→1Cer; GT_1, NeuAcα2→3Galβ1→3GalNAcβ1→4Gal(3←2αNeuAc8←2αNeuAc)β1→4Glcβ1→1Cer. The abbreviations used were according to IUPAC-IUB recommendations (22) for neutral glycosphingolipids and according to Svennerholm (23) for gangliosides.

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(Lakeside Lab.,Milwaukee,Wisc.) (10 mg/Kg) to inhibit monoamine oxidase (monoamine:O_2 oxidoreductase:EC 1.4.3.4.). After 3 h the corpus striatum was dissected from the brain and homogenized in 20 vol. of 0.3 M sucrose. A subcellular fraction containing synaptosomes was obtained by centrifugation between 1000-20000 x g_{av} for 20 min and the pellet resuspended (1.6 mg protein/ml) in a buffer-saline medium (145 mM-NaCl; 3.85 mM-KCl; 10 mM-glucose; 0.56 mM-ascorbic acid; 20 mM-Tris-HCl, pH 7.4). The loading and the determination of the release of neurotransmitter was essentially according to Holz (3). To the suspended synaptosomes [^{3}H]-dopamine at the final concentration of 0.13 µM was added and incubated at 37°C for 15 min. The mixture was cooled at 0-5°C and centrifuged at 20000 x g_{av} for 20 min. The pellet was briefly homogenized (6 mg protein/ml) in 0.3 M sucrose.

The lipids tested in the release experiments were previously dispersed by sonication for 2 min in the buffer-saline medium containing or not 1.5 mM-$CaCl_2$ and 0.65 mM-$MgSO_4$ as will be indicated. The determination of neurotransmitter release was initiated by adding 0.25vol.of the loaded synaptosomal preparation. The final concentration of added lipids was between 140-280 µM. The mixture was incubated at 37°C and aliquots containing 0.18 mg of protein were removed at different times and filtered through millipore membranes (0.45 µm pore size). The membranes were washed with 10 ml of the buffer-saline medium without bivalent cations and the radioactivity determined after dissolving the membranes in Bray's scintillation liquid.

Other methods and materials. Lactate dehydrogenase (EC 1.1.1.27.) was determined according to Kornberg (4) in samples containing 25 µg of protein/ml. When a mixture of triton CF54 and tween 80 was included in the system their final concentration was 0.9 and 0.45 mg/ml,respectively. Proteins were determined according to Lowry et al. (5). The glycosphingolipids were obtained as described previously (6).Other lipids were purchased from Sigma Chem.Co.(London, SW6,U.K.). [^{3}H-(G)]-Dihydroxyphenylethylamine (dopamine) sp.act. 6.45 Ci/mmol was from New England Nuclear (Boston, Mass U.S.A.).

RESULTS

The dopamine content of the synaptosomal preparation incubated for 15 min in the buffer-saline medium, with or without Ca^{++}, in the absence of added lipids was approx. 75 per cent of the value obtained at zero time (Fig. 1). When the incubation was carried out in the presence of Ca^{++} plus lipids capable of inducing permeability changes and membrane fusion in chicken erythrocytes such as glycerylmonooleate (7) or the polysialogangliosides GD_3, GD_{1a} and GT_1 (2) the dopamine remaining in the synaptosomes after 15 min of incubation decreased to values of 40-25 per cent of the original content. Chemically related lipids that were non-fusogenic in chicken erythrocytes such as glycerylmonostearate or the monosialoganglioside GM_1 or the neutral glycosphingolipid Gg_4Cer were ineffective to increase the release of dopamine from synaptosomes.

The induction of neurotransmitter release by these lipids had a requirement of Ca^{++}. In the absence of this metal the dopamine

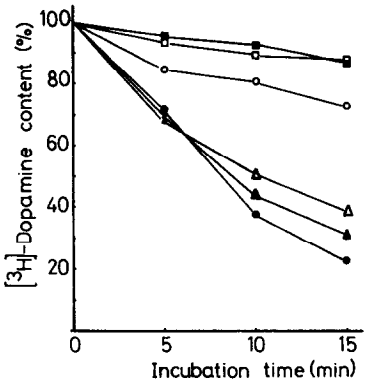

Fig.1. Release of [^{3}H]-dopamine induced by different lipids. The dopamine content is given as percentage of [^{3}H]-dopamine remaining in the synaptosomal preparation at different incubation times. The contents at zero time are taken as 100 per cent; this value represented about 40000 d.p.m. for each preparation. Each point is the average of between two and four different experiments. Reproducibility was about ± 4 per cent. The curves show the effects of : (○) no added lipid, plus Ca^{++}; (■) Gg_4Cer, 260 µM, plus Ca^{++}; (□) GM_1, 260 µM, plus Ca^{++} or GD_{1a}, 210 µM, without Ca^{++}; (△) GD_{1a}, 210 µM, plus Ca^{++}; (▲) GD_3, 210 µM, plus Ca^{++} or GT_1, 140 µM, plus Ca^{++}; (●) glyceryl-monooleate, 280 µM, plus Ca^{++}. Without Ca^{++}, glycerylmonooleate, 280 µM, or GD_3, 210 µM, or GT_1, 140 µM (not shown) gave values similar to the control.

contents of the preparations in which fusogenic lipids were added were in the level of the control or above at all times of incubation.

To discard the possibility that the increase of the release of dopamine was due to synaptosomal disruption we studied the activity of the cytosol enzyme lactate dehydrogenase. The values of lactate dehydrogenase activity measured after 15 min of incubation in the conditions used for neurotransmitter release were practically the same (0.42 units/mg protein) for control samples or for mixtures containing glycerylmonooleate or GD_{1a}. If in addition to the lipids triton CF54 plus tween 80 were added the values of lactate dehydrogenase increased to 1.0 units/mg protein, indicating disruption of the synaptosomal compartment (8).

DISCUSSION

The requirement for Ca^{++} and the lack of evidence of disruption of the synaptosomal compartment in the release of dopamine induced

by polysialogangliosides support that this process is mediated by fusion between the membrane of a neurotransmitter-containing vesicle and the plasma membrane of the nerve ending . This conclusion is also in agreement with the ability of polysialogangliosides to induce membrane fusion in chicken erythrocytes (2). Monosialo-gangliosides and neutral glycosphingolipids, on the other hand, did not induce fusion of chicken erythrocytes and were not able to increase the release of dopamine from synaptosomes. Gangliosides are synthesized in the neuronal perikarya and reach the synaptosomal plasma membrane, presumably through the process of axonal transport (9,10). Even if in the present work the gangliosides were added exogenously their effect may be indicative of the function of these compounds in nerve membranes since exogenous gangliosides can be incorporated in various cell membranes (11) including those of central nervous system (12). Moreover, in some cell systems it has been demonstrated that gangliosides added exogenously are incorporated in the membrane and can function as receptors, similarly to the corresponding endogenous glycolipids (13).

With respect to the molecular mechanisms underlying the effects involved in the exocytosis-mediated neurotransmitter release, there is evidence indicating that characteristic interactions with phospholipids in the membrane may be participating. Interactions between di- and tri-sialogangliosides and phosphatidylcholine in lipid monolayers occur with decrease of surface potential and modifications of molecular packing, a phenomenon also shown by other fusogenic compounds either lipidic or water-soluble (14). Chemically related compounds not able to induce membrane fusion did not have this type of interactions with phosphatidylcholine (15,16,17,18). If endogenous polysialogangliosides interact with phosphatidylcholine after a stimulus it may be expected that changes of surface potential and molecular packing similar to those found in lipid monolayers take place in the synaptosomal membrane. The membrane surface potential is a factor controlling ionic permeabilities (19,20) and the presence of gangliosides increases the conductance of phosphatidylcholine bilayers (21). Similarly to the effects of several other fusogenic agents in other systems (7,14) the membrane fusion induced by polysialogangliosides may involve modifications of the membrane organization accompanied by permeability changes and increased Ca^{++} entry into the nerve ending which would subsequently trigger the fusion process and result in neurotransmitter release.

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