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NEUROTRANSMITTER MOVEMENTS IN NERVE ENDINGS

INFLUENCE OF SUBSTANCES THAT MODIFY THE INTERFACIAL POTENTIAL

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Summary

Polysialogangliosides, sulphatides, glycerylmonooleate, unsaturated fatty acids, myelin basic protein and sucrose inhibit the Na^+-coupled uptake and induce a Ca^{2+}-dependent release of dopamine from nerve endings. Substances chemically related to those referred to above, such as monosialogangliosides, neutral glycosphingolipids, glycerylmonostearate, saturated fatty acids and albumin, do not show these effects. Mixtures of polysialogangliosides or sulphatides with myelin basic protein or albumin inhibit, to different degrees, the effects of the individual components. The decreased uptake induced by sucrose reverted to control levels upon reduction of the concentration of the perturbing agent. The restoration of the uptake was probably mediated by the Na^+-pump reconstituting the transmembrane Na^+-gradient necessary for the Na^+-coupled cotransport of dopamine. It is suggested that the effects of uptake inhibitor or release inducer agents derive from their ability to decrease the surface potential and modify the molecular organization of phospholipid interfaces which can result in changes of the membrane ionic permeability.

Abbreviations: tetrahexosylceramide, Galβ1 → 3GalNAcβ1 → 4Galβ1 → 4Glcβ1 → 1Cer; GD3, NeuAcα2 → 8NeuAcα2 → 3Galβ1 → 4Glcβ1 → 1Cer; GM1, Galβ1 → 3GalNAcβ1 → 4Gal (3 ← 2αNeuAc)β1 → 4Glcβ1 → 1Cer; GD1a, NeuAcα2 → 3Galβ1 → 3GalNAcβ1 → 4Gal (3 ← 2αNeuAc)β1 → 4Glcβ1 → 1Cer; GT1, NeuAcα2 → 3Galβ1 → 3GalNAcβ1 → 4Gal (3 ← 2αNeuAc8 ← 2αNeuAc)β1 → 4Glcβ1 → 1Cer. The abbreviations for gangliosides were according to Svennerholm [31].

Introduction

Release of neurotransmitter occurs by processes which include Ca^{2+}-dependent exocytosis in which the synaptic vesicle fuses with the synaptosomal plasma membrane [1]. It has been shown that polysialogangliosides induce a Ca^{2+}-dependent release of neurotransmitter from nerve endings [2] and this effect is coincident with the ability of these lipids to induce membrane fusion in chicken erythrocytes [3], a property shared by a considerable number of substances.

We will describe herein that several lipid- or water-soluble fusogenic substances, apart from gangliosides, are able to induce a Ca^{2+}-dependent neurotransmitter release. In addition, their effect on transmitter uptake is studied. From the results of the present work correlated with previous studies [4—9] it is suggested that both release and uptake of transmitter in nerve endings may be controlled at the molecular level by the dipolar organization adopted at the membrane interface under the influence of exogenous effectors.

Materials and Methods

Materials. Gangliosides GM1, GD1a, GD3, GT1 and tetrahexosylceramide were prepared as reported [10]. Sulphatides, cerebrosides and myelin basic protein were purified as described elsewhere [11]. Other lipids, bovine serum albumin (crystallized and lyophilized), dimethylsulphoxide and ouabain were from Sigma Chem. Co. (St. Louis, MO); sucrose was from B.D.H. (London), [G-^{3}H]dihydroxyphenylethylamine (dopamine) spec. act. 6.45 Ci/mmol was from New England Nuclear (Boston, MA).

Subcellular fraction. Female rats (200—250 g body weight) were injected intraperitoneally with pheniprazine (Lakeside Laboratories, Milwaukee, WI) (10 mg/kg). After 3 h the corpus striatum was dissected from the brain and homogenized in 20 vols. of 0.3 M sucrose. A subcellular fraction containing synaptosomes was obtained by centrifugation at $1000 \times g$ for 10 min, the supernatant was centrifuged at $20\,000 \times g$ for 20 min and the pellet resuspended (6.6 g protein/l) in a saline buffer medium (145 mM NaCl; 3.85 mM KCl; 0.65 mM $MgSO_4$; 10 mM glucose; 0.56 mM ascorbic acid; 20 mM Tris-HCl, pH 7.4).

Neurotransmitter uptake. 0.1 ml of the synaptosomal preparation was added to 0.4 ml of the saline buffer medium containing the substance to be tested at the concentration that will be indicated. The substances to be studied were dispersed by sonication for 2 min (Branson sonifier, 10—20 W output) in the saline buffer medium and the synaptosomal preparation was immediately added. The mixtures were preincubated for 5 min (Table II) or 10 min (Table I) at 37°C; [^{3}H]dopamine at the final concentration of 0.13 μM was added and aliquots containing approx. 0.13 mg of protein were subsequently filtered at 2.5 and 5 min through Millipore membranes (0.45 μm pore size). The membranes were washed with 10 ml of the saline buffer medium and the radioactivity remaining in the filter was determined by scintillation spectrometry using dioxane scintillation fluid. The concentration of dopamine is in the value of

the apparent K_m for the high affinity Na^+-coupled uptake system of dopaminergic synaptosomes [12].

Neurotransmitter release. The loading and determination of the release was essentially according to Holz [13]. To the synaptosomal preparation in the saline buffer medium (1.6 g protein/l) [^{3}H]dopamine at the final concentration of 0.13 μM was added and incubated at 37°C for 15 min. The mixture was centrifuged at 20 000 × *g* for 20 min. The pellet was homogenized (6 g protein/l) in 0.3 M sucrose. The transmitter release was initiated by adding 0.14 ml of the loaded synaptosomal preparation to 0.56 ml of the saline buffer medium with or without 1.5 mM $CaCl_2$ as indicated, including the substance to be tested dispersed by sonication as described above. The mixture was incubated at 37°C and aliquots containing about 0.18 mg of protein were removed at 0, 5, 10 and 15 min and filtered through Millipore membranes, washed and counted as indicated for neurotransmitter uptake.

Results

Neurotransmitter release. It has previously been reported that polysialogangliosides induce release of dopamine from synaptosomes in presence of Ca^{2+} [2]. This observation has not been extended to several lipids and water-soluble substances. The neurotransmitter remaining in the synaptosomal preparation after 15 min of incubation in the presence of the most effective agents ranged between 8—30% of the value at zero time (Fig. 1; Table I, group 1; Table II,

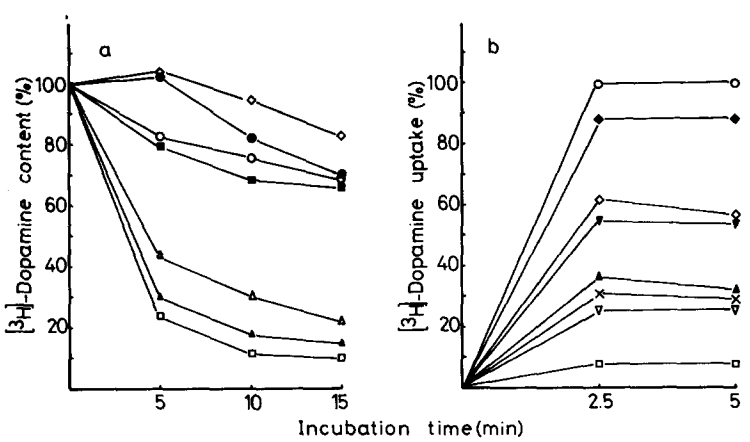

Fig. 1. Time dependence of dopamine movements induced by different effectors. For release (a), the dopamine content is the percentage of [^{3}H]dopamine in the synaptosomal preparation at different times, with respect to zero time which was about 2.8 pmol for each preparation. Reproducibility is given under Table I. The different preparations contained: ○, no effector; □, 3.9 M dimethylsulphoxide; ▲, 14 μM myelin basic protein; △, 160 μM GD1a; ●, 160 μM GD1a plus 8 μM myelin basic protein. All incubations were with 1.5 mM $CaCl_2$. ■, 3.9 M dimethylsulphoxide; ◇, 160 μM GD1a without $CaCl_2$. For uptake (b), the dopamine accumulation is given as the percentage of [^{3}H]dopamine taken up by the synaptosomal preparation without effector which was about 4.6 pmol and 5.3 pmol at 2.5 and 5 min of incubation, respectively. The different preparations contained: ○, no effector; ▽, 160 μM sulphatide; ▲, 8 μM myelin basic protein; ◇, 160 μM sulphatides plus 8 μM myelin basic protein; □, 0.9 M sucrose. For uptake curves labelled ◆, × and ▼, the conditions employed and the control taken as 100% are given in the legend to Table II for samples a, b and c, respectively.

TABLE I

DOPAMINE MOVEMENTS INDUCED BY LIPID EFFECTORS

The dopamine retained represents the [^{3}H]dopamine in the synaptosomal preparation after 15 min of the incubation with an effector and 1.5 mM $CaCl_2$ with respect to zero time, which was approx. 2.8 pmol for each preparation. For uptake, the values represent the [^{3}H]dopamine accumulated after 2.5 min of incubation with an effector, compared to the control which was approx. 4.6 pmol. The values are the average of two or more different experiments; reproducibility was approx. ± 4%. The time-dependence of all the effects is similar to those shown in Fig. 1. The numbers in brackets correspond to references.

	Effector (μM)	Dopamine retained (%)	Effector (μM)	Dopamine uptake (%)	Membrane fusion in chicken erythrocytes	Surface potential and packing in phosphatidyl-choline monolayers
No effector (control)	—	69	—	100	Negative	—
Group 1						
Glycerylmonooleate	280	23	280	32	Positive [8]	Decreased [6]
Oleic acid	280	12	280	14	Positive [8]	Decreased [6]
Palmitoleic acid	280	12	—	—	Positive [8]	Decreased [5]
Sulphatide	280	20	280	18	Positive [9]	Decreased [9]
GD3	210	32	—	—	Positive [3]	Decreased [4]
GD1a	210	23	280	28	Positive [3]	Decreased [4]
GT1	140	23	—	—	Positive [3]	Decreased [4]
Glyceryldioleate	570	51	280	97	Weak [8]	Decreased [5] *
Group 2						
Glycerylmonostearate	280	109	280	84	Negative [8]	no change or increased [6]
Stearic acid	280	82	280	105	Negative [8]	no change or increased [6]
Palmitic acid	280	81	—	—	Negative [8]	no change or increased [5]
Cerebroside	280	76	160	82	Negative [9]	no change or increased [9]
Tetrahexosylceramide	260	88	—	—	Negative [3]	no change or increased [4]
GM1	260	88	280	123	Negative [3]	no change or increased [4]
Glyceryldipalmitate	570	84	280	108	Negative [8]	no change or increased [5]

* The change is dependent on the type of fatty acyl chain of the phospholipid.

TABLE II

DOPAMINE MOVEMENTS INDUCED BY WATER-SOLUBLE AND LIPID PROTEIN MIXTURES

The general conditions employed, amounts of dopamine taken as 100% for controls, and reproducibility are as given in Table I. The time-dependence of all the effects is similar to that shown in Fig. 1. (a) The preparation was incubated for 5 min at 37°C in saline buffer 0.9 M sucrose, then diluted 4-fold with saline buffer and incubated for 10 min and the [^{3}H]dopamine was added. (b) Same as in (a) but the dilution step was done with a saline buffer 1.33 μM ouabain. (c) The preparation was incubated for 5 min at 37°C in a saline buffer, then diluted 4-fold with saline buffer 0.31 M sucrose 1.33 μM ouabain, and incubated for 10 min, and the [^{3}H]dopamine was added. As control for (a), (b) and (c), the preparation was incubated for 5 min at 37°C in a saline buffer, then diluted 4-fold with saline buffer 0.31 M sucrose, incubated for 10 min, and the [^{3}H]dopamine was added. Uptake in this sample was about 70—80% of a control in which the dilution was done with a saline buffer without sucrose.

	Effector (μM)	Dopamine retained (%)	Effector (μM)	Dopamine uptake (%)	Membrane fusion in chicken *erythrocytes*	Surface potential and packing in *phosphatidylcholine* monolayers
No effector (control)	—	69	—	100	Negative	—
Group 1						
Myelin basic protein	8	14	8	36	Positive [9]	—
Sulphatide	160	20	160	26	Positive [9]	Decreased [9]
GD1a	160	23	160	26	Positive [3]	Decreased [4]
Sulphatide + myelin basic protein	160 : 8	37	160 : 8	62	Negative [9]	—
Sulphatide + albumin	160 : 8	21	160 : 8	57	Positive [9]	—
GD1a + myelin basic protein	160 : 8	70	160 : 8	41	—	—
GD1a + albumin	160 : 8	112	160 : 8	67	—	—
Group 2						
Myelin basic protein	8	14	15	12	Positive [9]	—
Albumin	8	94	8	92	Negative [9]	—
Dimethylsulphoxide	3.9×10^6	8	—	—	Positive [8]	Decreased [21]
Sucrose	9.0×10^5	13	$9.0 \cdot 10^5$	8	Positive [8]	Decreased [21]
Sucrose (a)	—	—	$9.0 \cdot 10^5$	88	—	—
Sucrose (b)	—	—	$9.0 \cdot 10^5$	31	—	—
Ouabain (c)	—	—	1	55	—	—

group 2). By contrast, the dopamine remaining in preparations incubated for the same period in the presence of other compounds chemically related to those referred to above (Fig. 1; Table I, group 2) was between 70—100% of the value at zero time and at the level of the control. These were also the values obtained in the presence of every compound if Ca^{2+} were omitted in the incubation medium. Values for the control were identical with or without Ca^{2+}. The free dopamine retained in the filter in control medium without synaptosomes is less than 2% of the amount retained in control samples incubated with synaptosomes.

When the individually efficient inducers of dopamine release, GD1a and myelin basic protein, were mixed and sonified together their individual effect was inhibited and the release was at the level of the control. A similar result was found for the mixture of GD1a plus albumin. With sulphatides, however, only about a 50% inhibition was obtained in a mixture with myelin basic protein and none with albumin (Table II, group 1). These results show that lipid-protein interactions can affect the behaviour of the individual components on the transmitter release. In this connection, it is relevant that the myelin basic protein shows preferential interactions with sulphatides in model systems [14]. Likewise, gangliosides from synaptosomes can be isolated associated tightly with protein [15] and the myelin basic protein can interact with liposomes containing sulphatides or gangliosides as evidenced by an increase of their permeability to glucose [16].

Neurotransmitter uptake. The most effective substances for inducing dopamine release caused amine uptakes of only 10—30% of the control (Fig. 1; Table I, group 1; Table II, group 2) whereas transmitter uptakes of between 80—100% of the control were obtained with other chemically related substances that were ineffective in inducing dopamine release (Fig. 1; Table I, group 2).

Similar to results obtained from release experiments, if sulphatides or gangliosides were mixed with myelin basic protein their individual effect was reduced. Albumin was also able to inhibit partially the effect of the lipids (Table II, group 1).

The inhibition of dopamine uptake induced by sucrose was reversible. A synaptosomal preparation incubated for 5 min with 0.9 M sucrose before the addition of the labelled transmitter showed 8% of the uptake with respect to the control (Table II, group 2). However, if before adding the [^{3}H]dopamine the preparation was diluted with the saline buffer medium to 0.23 M sucrose and incubated for a further 10 min, an accumulation of 88% of the control was obtained (Table II, group 2). If the dilution of sucrose was performed with a medium containing 1 μM ouabain (final concentration) to inhibit partially the Na^+-pump, only 31% of uptake was obtained. This value represents only 56% recovery of the uptake capacity (instead of 88% as above) when compared to the uptake of 55% for a preparation in which 1 μM ouabain was present but the 0.9 M sucrose in the initial step was omitted. The results suggest that the recovery of the uptake capacity in our preparation was mediated by the functioning of the Na^+-pump reconstituting a transmembrane Na^+-gradient that has been reduced or eliminated by an increased permeability induced by high sucrose. Reduced uptake [12] and increased Ca^{2+}-dependent release [17] of

transmitters by the presence of a high sucrose content were previously reported.

Discussion

The inhibition of dopamine uptake, brought about by the same agents that were the most efficient inducers of dopamine release, cannot be attributed to the last effect mentioned above since the release requires the presence of Ca^{2+} and this was omitted in the incubation mixtures used for the uptake determinations to avoid the possibility of a concomitant release. The presence of Ca^{2+} has practically no effect on uptake [12]. Conversely, the enhanced release observed with the effective uptake inhibitors was not the consequence of an impaired re-uptake because of the Ca^{2+}-dependence of the release effect. On the other hand, the possibility that the effects could be ascribed to disruptions of the synaptosomal compartment is ruled out because the activities of the cytosol enzyme lactate dehydrogenase in the experimental and control samples were similar [2,18], no effect on the release was found in absence of Ca^{2+} and the uptake was restored after dilution of a water-soluble agent that was inhibitory at high concentrations. All these results also appear to rule out the possibility that the amount of nerve endings retained in the filter may vary in the different conditions.

The series of compounds able to induce release of dopamine or to inhibit its uptake is considerably heterogeneous. It includes water-soluble substances and lipids such as different fatty acids, glyceryl esters and glycosphingolipids. Moreover, chemically related compounds do not behave in a similar way in this respect. Apparently, their effect is not the consequence of a common chemical structure. Nevertheless, an interpretation of their molecular mode of action can be proposed by correlating the results described in the present work with known effects of most of these substances in other natural and model membrane systems (Tables I and II). Compounds that, in the present work, are shown to affect neurotransmitter movements, but not their ineffective chemical analogues, are capable of inducing Ca^{2+} entry [8,19,20] and Ca^{2+}-dependent membrane fusion in chicken erythrocytes [3,8,9]. A further common property of substances acting as release inducers and uptake inhibitors is the ability to decrease the surface potential and molecular packing of phospholipid interfaces [4—9]. This behaviour is not found with chemically similar substances that do not affect transmitter movements. The modification of the interfacial properties of membrane lipids by the effective lipid substances can be accomplished fundamentally through ion-dipole interactions with choline-containing phospholipids [4—9]; effective water-soluble agents can have a similar action through changes in the aqueous environment of phospholipid head groups with the consequent modification of dipolar properties, packing, fluidity and permeability [20—22].

Ionic permeabilities are greatly influenced by the membrane surface potential which, in turn, is dependent on the dipolar organization of the surface [23—25]. The decrease of surface potential and altered membrane organization induced by the effective substances may thus explain their effects on transmitter uptake and release on the basis of altered ion movements down

their electrochemical gradients. Calcium is one such ion and its entry is an important requirement for triggering membrane fusion processes [8,9,19—21] and neurotransmitter release [1,2,13]. Uptake of transmitter by a Na^+-coupled cotransport requires a proper maintenance of low membrane permeability to Na^+ so that the energy stored in the transmembrane Na^+-gradient can be utilized for the transmitter transport process [26]. An enhanced permeability of the nerve ending membrane will allow an increased Na^+-influx which may depolarize the membrane and decrease the uptake of neurotransmitter. The results obtained in the present work for preparations allowed to 'recover' by incubation in the presence of diluted sucrose suggest that if the concentration of the perturbing agent is reduced the functioning of the Na^+-pump may reestablish an adequate transmembrane Na^+ gradient and Na^+ influx. This interpretation is supported by an impaired restoration of the uptake in these conditions when ouabain is present (Fig. 1; Table II, group 2).

When several observations related to transmitter movements induced by chemical effectors or physiological stimuli are taken together, they show that the initial effect must involve modifications of interactions between membrane associated molecules affecting the permeability steady state of the membrane. With naturally occurring membrane constituents, such as polysialogangliosides, sulphatides and myelin basic protein, the diminution of their individual effect when the lipids and proteins were mixed (Table II, group 1) suggests that lipid-protein interactions can play a role in controlling the availability in the membrane of a release inducer or uptake inhibitor compound. Recently it has been reported that phospholipase A_2 and β-bungarotoxin enhance the release and depress the uptake of transmitters through modifications of the membrane potential as evidenced by cyanine dyes [27]; the toxin also exhibits phospholipase A_2 activity [28] and will produce lysophospholipids and free unsaturated fatty acids which are effective in provoking permeability changes and membrane fusion (cf. Ref. 8). Moreover, unsaturated fatty acids added to preparations of nerve endings depress the uptake and stimulate the release of transmitters [18,27]. These effects can probably be understood within the general explanation proposed above on the basis of modified intermolecular interactions in the membrane.

Apart from channels [25,29,30] and modifications of surface dipoles and electrical fields at the membrane interface [4—9,21,22,29], which participate in ion and transmitter movements, the correlations discussed herein point to decreases in the surface potential of membrane phospholipids as an initial step to be considered for triggering these processes.

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