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SOME PHARMACOLOGICAL PROPERTIES OF BRAIN GANGLIOSIDE

BY

S. BOGOCH, M. K. PAASONEN,* AND U. TRENDELENBURG

From the Neurochemical Research Laboratory† (Department of Psychiatry) and the Department of Pharmacology, Harvard Medical School, Boston, Mass., U.S.A.

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The effects of a preparation of purified brain ganglioside and of neuraminic acid have been studied on various pharmacological test preparations. In concentrations of 10 μg/ml. or more, ganglioside stimulates the isolated guinea-pig ileum; graded responses are usually obtained. Various substances, known to antagonize the actions of other stimulant substances, fail to affect the response to ganglioside. Ganglioside and neuraminic acid neither stimulate the superior cervical ganglion of the cat nor do they affect ganglionic transmission. Ganglioside and neuraminic acid did not change the response of the frog rectus abdominis muscle to acetylcholine, nor did they affect the blood pressure of the cat. Ganglioside (2 μg/ml. or more) stimulates the isolated heart of *Venus mercenaria*, but the response, which develops slowly, decreases with repeated administration of ganglioside. The response is not blocked by 2-bromo-lysergic acid diethylamide and there is no indication that it is due to the liberation of endogenous 5-hydroxytryptamine or related indole compounds. For both the heart of *Venus mercenaria* and guinea-pig ileum, the activity demonstrated by brain ganglioside is specifically a property of the whole molecule, since neither neuraminic acid itself nor a preparation of brain ganglioside from which only two-thirds of its neuraminic acid had been removed are active.

Ganglioside is a macromolecular glycolipid present in the grey matter of brain. The chemical structure of the repeating chain of a preparation of bovine brain ganglioside has recently been formulated by Bogoch (1958) and confirmed by Klenk & Gielen (1960). Nothing is known about the physiological role of this and of related compounds, but various pharmacological actions of this substance have been described. Thus, brain ganglioside inhibits the viral hemagglutination reaction (Bogoch, 1957) and the neurotoxic effects of two types of influenza virus in mouse brain *in vivo* (Bogoch, Lynch & Levine, 1959); it binds tetanus toxin *in vitro* (van Heyningen, 1959a, b, c); it stimulates the isolated heart of *Venus mercenaria* (Bogoch & Bogoch, 1959) as well as the isolated intestine of the rabbit (Vogt, 1960). Balakrishnan & McIlwain (1961) have also demonstrated that brain ganglioside is effective in restoring the ability of brain slices to respond to electrical impulses.

The present work was carried out in order to obtain more information about the pharmacological actions of a purified preparation of brain ganglioside and in order

* Present address: Department of Pharmacology, University of Helsinki, Finland.

† Now: Foundation for Research on the Nervous System, 36 The Fenway, Boston, Mass., U.S.A.

to analyse its mode of action. All of the members of the family of macromolecular glycolipids in the nervous system, structurally related to bovine brain ganglioside, and termed aminoglycolipids (Bogoch, Faillace, Belval & Winer, 1961), have not yet been studied pharmacologically. The structure of the basic repeating unit of the preparation of bovine brain ganglioside here tested is known to be N-acetyl neuraminic acid–N-acetyl galactosamine–galactose–glucose–sphingosine–stearic acid (Bogoch, 1958; Klenk & Gielen, 1960). The hydrolytic removal of the neuraminic acid moiety permitted testing of its pharmacological activity as well as that of the balance of the molecule remaining after its removal.

METHODS

Isolated guinea-pig ileum. Pieces were taken from the terminal part of the ileum of freshly killed animals and suspended in aerated Tyrode solution at 35° C. The bath volume was 2.5 ml. The responses were recorded by means of an isotonic lever fitted with a frontal writing-point.

Isolated rectus abdominis muscle of the frog. This muscle was suspended in aerated frog-Ringer solution in a 2.5 ml. bath at room temperature of about 25° C.

Ganglionic transmission. After induction of anaesthesia with ether, spinal cats were prepared as described by Burn (1952). The movements of the nictitating membrane were recorded with an isotonic lever fitted with a frontal writing-point; the weight on the nictitating membrane was 7 g, and the contractions were magnified 12.4 times. The blood pressure was recorded from the left femoral artery. Intra-arterial injections into the blood supply of the right superior cervical ganglion were made retrogradely through the lingual into the common carotid artery (Trendelenburg, 1959). During occlusion of the external carotid artery such injections reach the ganglion as verified by its immediate response to intra-arterial injections of small amounts of nicotine (10 to 50 μg). For preganglionic stimulation the cervical sympathetic chain was cut and its peripheral end was placed on bipolar platinum electrodes and covered with liquid paraffin. A conventional electronic stimulator (Grass) producing rectangular impulses of 1 msec duration was used. The rate of stimulation was 25 shocks/sec and the voltage was adjusted to obtain submaximal responses of the nictitating membrane.

Arterial blood pressure. Cats were anaesthetized with sodium pentobarbitone, 35 mg/kg, and kept under artificial respiration. The mean arterial blood pressure was recorded from the carotid artery with a mercury manometer.

Isolated heart of Venus mercenaria. The spontaneously beating isolated heart was suspended in an overflow bath perfused with sea-water containing 6 mg/l. benzoquinonium (Paasonen & Giarman, 1958).

The ganglioside used was isolated from bovine brain in its native salt form, and N-acetyl neuraminic acid was isolated therefrom (Bogoch, 1958). This brain ganglioside had been passed through six repartitions with chloroform:methanol:water in order to free it of the closely related non-dialysable hexosamine- and hexose-rich aminoglycolipids recently recognized in association with brain ganglioside (Bogoch *et al.*, 1961).

With acetylcholine chloride, histamine acid phosphate and 5-hydroxytryptamine creatinine sulphate, the weights refer to the free bases. With all other substances, the weights refer to the salts.

RESULTS

Guinea-pig ileum. Ganglioside caused a contraction of this preparation, the threshold concentration being about 10 μg/ml. Five times higher concentrations always caused a response which usually consisted of an increase in tone but frequently also of an increase in the amplitude of spontaneous movements. The response reached its maximum within 20 to 45 sec. After washing it could be

repeated at intervals of 3 min, but the responses of some preparations were rather variable. However, graded responses were usually obtained, and an increase in concentration by 30% was detectable. During the course of an experiment the responses were likely to increase as the spontaneous activity increased.

The influence of various substances on the response of the ileum to ganglioside was studied by adding these substances to the bath after each washing and by leaving them in contact with the ileum for periods of 10 to 30 min ; during this time the response to ganglioside and to other substances was tested.

Atropine sulphate in concentrations of 0.001 to 0.003 μg/ml. reduced the response to ganglioside by 20 to 40% in 5 of 7 experiments. The response to acetylcholine, though of greater magnitude than that to ganglioside, was inhibited by at least 75% in all experiments. When the response to ganglioside was inhibited by atropine, the spontaneous activity of the ileum was also lowered and the response to histamine and nicotine was reduced slightly.

Mepyramine maleate (0.005 μg/ml., 3 experiments) and chlorprophenpyridamine maleate (0.5 to 1.5 μg/ml., 3 experiments) clearly antagonized histamine, but had no obvious effect on the response to ganglioside. Cocaine hydrochloride (10 μg/ml., 4 experiments) had no consistent effect on the response of the ileum to ganglioside and, if any, affected similarly the response to histamine. Hexamethonium chloride (20 μg/ml., 4 experiments) reduced the response to nicotine by 50 to 75%, but the response to ganglioside was unaltered or slightly increased, as was the spontaneous activity of the ileum. Dihydroergotamine methane sulphonate (2 μg/ml., 4 experiments) reduced the response to ganglioside by 30 to 60%. The responses to histamine, acetylcholine and nicotine were reduced to about the same extent by this dose. Papaverine hydrochloride (2 to 20 μg/ml., 4 experiments) abolished the response of the ileum to ganglioside, histamine, acetylcholine and nicotine. Ganglioside itself (in doses from 1 to 100 μg/ml.) did not affect the response of the ileum to histamine and acetylcholine when it was left in the bath for 20 to 30 min. In 2 of 5 experiments there was a slight inhibitory effect of ganglioside (100 μg/ml.) on the response to 5-hydroxytryptamine. The dose-response curve for 5-hydroxytryptamine, however, was flat ; the response to nicotine was not affected when the response to 5-hydroxytryptamine was found to be reduced.

Neuraminic acid in concentrations of up to 250 μg/ml. had no effect on the isolated guinea-pig ileum (17 experiments). The stimulant effect of ganglioside on the guinea-pig ileum was lost when two-thirds of the neuraminic acid moieties were removed by autohydrolysis at 100° C (residue A, Bogoch, 1958).

Transmission through the superior cervical ganglion. After determination of the responses of the nictitating membrane to supramaximal preganglionic stimulation (applied for periods of 5 sec each every 30 or 60 sec), the voltage was reduced until contractions were obtained, the magnitude of which was 20 to 50% of that of the maximal response. Intra-arterial injections to the superior cervical ganglion of ganglioside (varying from 5 μg to 1 mg) failed to exert any influence on the ganglion or on the transmission of submaximal preganglionic impulses through the ganglion (9 observations). Time intervals of up to 30 min were allowed between injections so as to avoid " tachyphylaxis." Similarly negative results were obtained

with intra-arterial injections of neuraminic acid into blood supply of the superior cervical ganglion. This substance (in doses from 1 to 200 μg, 9 observations) neither stimulated the ganglion nor facilitated or depressed ganglionic transmission. The preparations used for these experiments responded well to the usual dose of nicotine (10 μg), histamine (5 μg) or pilocarpine (10 μg).

Blood pressure of the cat. In four experiments the intravenous injection of 500 μg/kg of ganglioside and of 100 μg/kg of neuraminic acid had no influence on the arterial mean blood pressure.

Frog rectus abdominis muscle. Ganglioside and neuraminic acid failed to cause a response of this muscle when left in the bath for 1 to 3 min in concentrations up to 200 μg/ml. (ganglioside) or 40 μg/ml. (neuraminic acid) (4 preparations). The two substances did not affect the response of the muscle to acetylcholine when added to the bath 60 to 90 sec prior to the acetylcholine and when left in the bath while acetylcholine exerted its normal action. In the presence of 10 μg/ml. of physostigmine salicylate, ganglioside likewise failed to modify the response to subsequent addition of acetylcholine.

Isolated heart of Venus mercenaria. In concentrations of 1 μg/ml. and less, ganglioside had no effect (12 preparations), but concentrations of 2 to 5 μg/ml. or more usually increased the rate and amplitude of the spontaneous heart beat. In a concentration of 100 μg/ml. ganglioside had a pronounced stimulant effect, but when left in the bath for 3 to 10 min an inhibitory effect was apparent, and the rate of heart beat became irregular. In contrast to the results obtained with the isolated guinea-pig ileum, the response of the heart of *Venus mercenaria* to ganglioside was observed only after the first administration or the response was smaller when the same concentration of ganglioside was applied again. The normal response to ganglioside was not immediate but developed after a latency period of 15 to 60 sec.

5-Hydroxytryptamine is known to stimulate the clam heart in very low concentrations (Welsh, 1953; Paasonen & Giarman, 1958). Since 5-hydroxytryptamine is known to be present in the heart of *Venus mercenaria* (Welsh & Moorhead, 1960), the following experiments were carried out to test for a possible release of endogenous 5-hydroxytryptamine by ganglioside: 20 μg/ml. of 2-bromo-(+)-lysergic acid diethylamide, which completely blocked the response to 5-hydroxytryptamine (Welsh & McCoy, 1957), was present in the bath before the administration of 100 μg/ml. of ganglioside. In four experiments the response to ganglioside did not differ from that observed in the absence of 2-bromo-(+)-lysergic acid diethylamide.

The heart of *Venus mercenaria* is also sensitive to N-N-dimethyl-5-hydroxytryptamine and to bufotenin (Bumpus & Page, 1955), but the response to these substances can be differentiated pharmacologically from that to 5-hydroxytryptamine (Paasonen & Giarman, 1958). Aqueous extracts of hearts of *Venus mercenaria* and extracts obtained with 95% acetone did not contain any activity resembling that of the two 5-hydroxytryptamine derivatives. Furthermore, 2-bromo-(+)-lysergic acid diethylamide also abolished the response of the heart to these indoles.

When ganglioside (1 to 100 μg/ml.) was left in the bath for 5 to 10 min, the response to the subsequent addition of 5-hydroxytryptamine to the bath was not

altered. Acetylcholine inhibits the heart in very low concentrations. In four experiments (with no benzoquinonium in the perfusate) the threshold concentration of about 0.0005 μg/ml. of acetylcholine was not changed by the presence of ganglioside in concentrations of up to 100 μg/ml.

DISCUSSION

Brain ganglioside has been found to stimulate the isolated guinea-pig ileum. Experiments with specific inhibitors indicate that acetylcholine and histamine receptors are not involved in the response of the ileum to ganglioside. That the receptors for histamine, acetylcholine and 5-hydroxytryptamine are not involved is also indicated by the observation that ganglioside itself did not change the response of the ileum to these substances. The site of action of ganglioside differs from that of 5-hydroxytryptamine, since dihydroergotamine had not the specific effect against ganglioside which it is known to have against 5-hydroxytryptamine (Gaddum & Picarelli, 1957). The failure of cocaine and of hexamethonium to change the response of the ileum to ganglioside is an indication that the nervous elements of the ileum are not involved in this response. Whenever a change in the response to ganglioside was produced by the administration of any of these substances, a parallel change was observed in the response to other biologically active substances, whose sites of action are believed to differ from each other.

Direct evidence for the lack of effect of ganglioside on at least some autonomic ganglia was obtained in experiments in which this substance and neuraminic acid were injected into the blood supply of the superior cervical ganglion of the cat. Both substances failed to stimulate the ganglion or to modify ganglionic transmission ; they also had no action on a neuromuscular junction, since they failed to modify the response of the frog rectus abdominis muscle to acetylcholine. On the other hand, the activity of ganglioside in brain slices (Balakrishnan & McIlwain, 1961) and on frog sciatic nerve (Trams & Spyropoulos, personal communication) demonstrates that ganglioside has an effect at certain sites of the nervous system.

The results obtained on the isolated heart of *Venus mercenaria* indicate that the slowly developing response of this heart to ganglioside is not due to the release by ganglioside of endogenous 5-hydroxytryptamine or of other related hydroxyindoles. It is unlikely to be due to the liberation of histamine or of catecholamines, because these compounds are much less effective on this tissue (Greenberg, 1960). Neither the stimulant effect of 5-hydroxytryptamine nor the inhibitory effect of acetylcholine was changed by ganglioside. While brain ganglioside is not known to be a native constituent of any of the tissues here tested, its activity in *Venus mercenaria* heart and in guinea-pig ileum, and its lack of activity in other preparations (frog rectus abdominis and superior cervical ganglion of the cat), indicate some selectivity of pharmacological action the basis of which is at present obscure.

The mechanism of action of brain ganglioside is unknown. Because of the presence of both water- and lipid-soluble groups, and the demonstration of viral receptor properties, a function of brain ganglioside at interphases (membrane, receptor and transmission functions in the nervous system) has been suggested (Bogoch, 1957, 1958 ; Bogoch *et al.*, 1959 ; Bogoch & Bogoch, 1959). The acidic groups of both phospho- and glyco-lipids form salts with cations. These salts are

water- and lipid-soluble and it has been suggested that their action on smooth muscle is due to an increased permeability to the bound cations as compared to the free ones (Vogt, 1958, 1960). That the neuraminic acid of ganglioside exerts its action simply as a binder of cations is not supported by the fact that the free neuraminic acid itself, which is also able to bind cations through the ionizable carboxyl group, is inactive alone. On the other hand, brain ganglioside from which two out of three of the terminal neuraminic acid residues have been removed (i.e., residue A, Bogoch, 1958) is also totally inactive. Thus, while the terminal neuraminic acid constituent is necessary for the activity, it is not sufficient; the activity is a property of the whole intact brain ganglioside. The fact that residue A, comprising 80% of the whole molecule with the bulk of both carbohydrate and lipid constituents still present in covalent linkage, is inactive also strongly supports the specificity of the stimulatory function of the intact molecule.

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