EFFECT OF XENON, NITROUS OXIDE AND HALOTHANE ON MEMBRANE-BOUND SIALIDASE FROM CALF BRAIN

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1. Introduction

Substances like xenon, nitrous oxide and halothane interact with proteins or membranes: xenon can be bound to hydrophobic regions of myoglobin [1]. Under hyperbaric pressures xenon, as well as nitrous oxide inhibit enzymes like tyrosinase, α-chymotrypsin and acetylcholinesterase [2]. On the other hand, halothane increases the fluidity of lipid membranes [3,4]. We studied the influence of these substances on membrane-bound sialidase which, together with its ganglioside and glycoprotein substrates, is found in neuronal membranes and mostly in the plasma membrane of nerve endings [5,6].

Unexpectedly we found an increase of particulate sialidase activity in the presence of higher concentrations of these substances when measured both on endogenous substrates as well as on the tritium-labelled ganglioside G_{D1a}*, added to the reaction mixture. This ganglioside is split by the enzyme into sialic acid and ganglioside G_{M1} [7,8]. In this paper we report the first observation of an increase in enzyme activity as a result of the addition of xenon, nitrous oxide and halothane. Other membrane-bound enzymes (5′-nucleotidase, adenyl cyclase and Na^+-K^+-ATPase) studied were slightly inhibited under comparable conditions.

*Abbreviations: Ganglioside G_{D1a} = AcNeu-(α2→3)Gal-(β1→3)GalNAc-(β1→4) [AcNeu-(α2→3)] Gal-(β1→4)Glc-(β1→1′)ceramide; ganglioside G_{M1} = Gal-(β1→3)GalNAc-(β1→4) [AcNeu-(α2→3)] Gal-(β1→4)Glc-(β1→1′)ceramide.

2. Materials and methods

2.1. *Preparation of particulate sialidase*

A 10% homogenate of fresh calf brain in 0.32 M sucrose was centrifuged at 13 000 *g* for 30 min and the microsomal pellet was sedimented at 150 000 *g* for 1 h. The microsomal pellet was washed twice with 10 mM Tris buffer, pH 7.4, in order to remove the sucrose. Aliquots of the final pellet were stored at −20°C and used within 14 days as particulate enzyme preparation.

2.2. *Sialidase assay with ganglioside G_{D1a}[^{3}H]*

The incubation mixtures were prepared at 0°C in polyethylene tubes which fitted tightly into 30 ml autoclaves. The incubation volume of 200 μl contained 12 nmol ganglioside G_{D1a}[^{3}H] (83 Ci/mole, labelled by reduction of the double bond in the sphingosine moiety with tritium gas [9], 30 μmol sodium acetate buffer, pH 4.2 and 200 μg of microsomal protein. Blanks were run with microsomes treated for 15 min at 20°C with 1.5% trichloroacetic acid. The mixtures were sonically irradiated twice for 10 sec each before adding the microsomes. For the effects of the gases the air was not excluded but was supplemented by xenon, nitrous oxide, hydrogen, neon, nitrogen or helium at pressures ranging from 2.5 up to 30 atmospheres. The incubation mixtures were shaken for 60 min at 35°C and the reaction stopped by cooling down to 0°C. After release of the gas the amount of ganglioside G_{M1} [^{3}H] formed was determined [9]: 40 μl of the incubation mixture were subjected to thin-layer chromatography on Silica gel-G-plastic sheets using the solvent system butanol–

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pyridine–0.55% KCl (6:3:2). The spots of product and substrate were localized by means of a radioscanner and were cut out. Their radioactivity was measured in a liquid scintillation counter. The standard deviation of the overall method is ± 6%.

2.3. *Assay of sialidase activity against endogenous substrates*

The incubation volume of 200 μl contained microsomes (800 μg of protein) and 30 μmol sodium acetate buffer, pH 4.2. The mixtures were incubated for 60 min at 37°C under the conditions outlined under 2.2. Blanks were run with microsomes treated for 15 min at 20°C with 1.5% trichloracetic acid. Sialic acid released was measured with the thiobarbituric acid test [10].

3. Results

Fig.1 shows the effect of six gases, at pressures ranging from 0 to 30 atmospheres on the hydrolysis of the G_{D1a}-ganglioside by a microsomal preparation obtained from calf brain. Higher reaction rates were obtained in the presence of nitrous oxide and xenon, but not upon the addition of helium, hydrogen, neon or nitrogen. Fig.1 also shows that halothane and the non-ionic detergent Triton X-100 increased the reaction rates at atmospheric pressure. Ultrasonic irradiation of the enzymatic preparation prior to exposure to the gases did not significantly alter the above results.

Similar results were obtained when measuring the release of sialic acid from the endogenous substrates (fig.2). Nitrous oxide and xenon at 30 atmospheres resulted in a five-fold increase of membrane-bound sialidase activity. Helium, hydrogen, neon or nitrogen at pressures up to 30 atmospheres did not have any effect on sialidase activity. The figure also shows that release of sialic acid from the endogenous substrates increased at atmospheric pressure in the presence of halothane or the detergent Triton X-100, and that high pressures of nitrous oxide or xenon did not further increase the sialidase activity of the microsomes when incubated in the presence of Triton X-100.

Three other membrane-bound enzymes that were tested, 5′-nucleotidase [11], Na^+-K^+-ATPase [12] and adenyl cyclase [13,14], were not stimulated but rather were slightly inhibited by xenon at 30 atmospheres (up to 40% inhibition). For the sake of comparison, the influence of these gases on the activity of a soluble enzyme was also examined. This enzyme, *N*-acetylhexosaminidase, was not affected by xenon at 30 atmospheres when using the water soluble substrate 4-methyl-umbelliferyl-*N*-acetyl-β-D-glucosaminide [15].

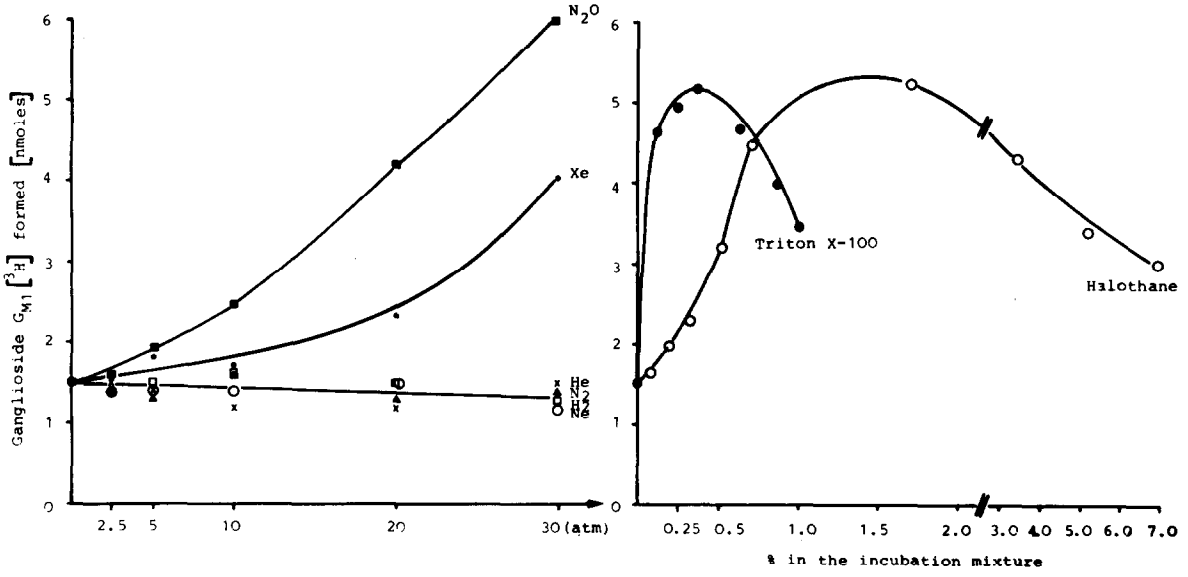

Fig.1. Effect of gases on membrane-bound sialidase activity against ganglioside G_{D1a}[^{3}H].

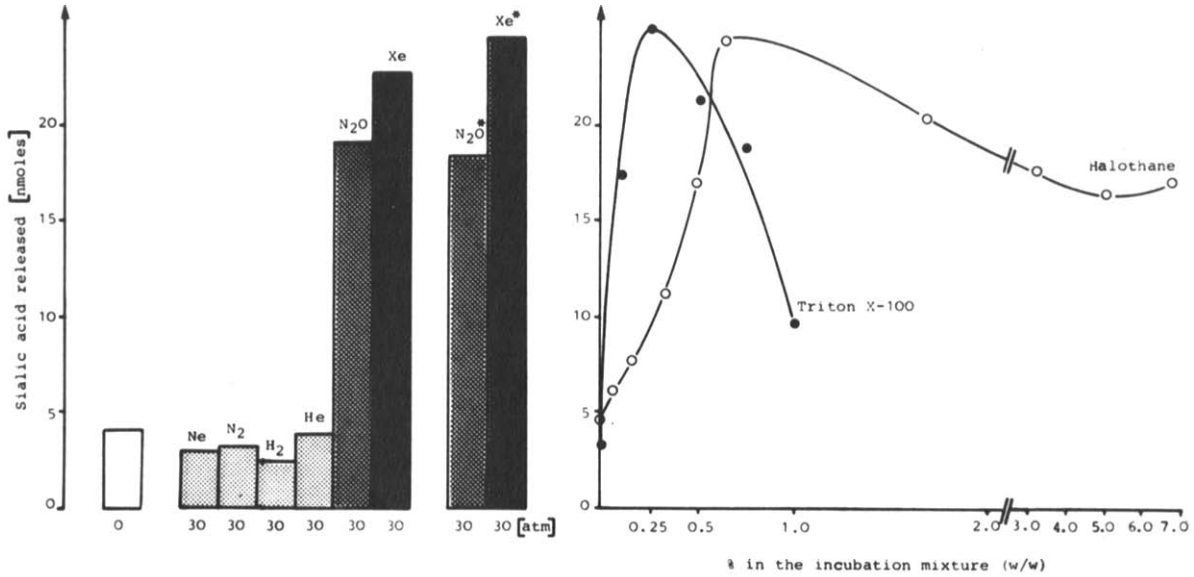

Fig.2. Effect of gases on membrane-bound sialidase activity against endogenous substrates. *Incubations were performed in the presence of 0.25% Triton X-100.

4. Discussion

Unexpectedly membrane-bound sialidase activity is not, in contrast to other enzymes [2], inhibited but rather enhanced by halothane and elevated pressures of xenon and nitrous oxide. In particular halothane exerts an effect on the enzyme similar to that of Triton X-100: low concentrations of the detergent enhance whereas higher concentrations inhibit the enzyme activity [16].

The mechanism by which xenon and nitrous oxide, in contrast to other gases (helium, neon, hydrogen and nitrogen) enhance particulate sialidase activity under elevated pressures cannot as yet be assessed. The finding that xenon and nitrous oxide exhibited no effect on the enzyme when assayed in the presence of Triton X-100 could suggest that both gases act primarily on the membrane structure. Solubilization of these gases in the hydrophobic phase of the membrane according to Meyer [17] and Overton [18] causes an increased fluidity of the membrane [4] which may render sialidase more active. Triton X-100 may exert its stimulating effect by a disintegration of the membrane structure.

The results suggest a regulatory function of normal membrane structure in controlling the activity of membrane-bound sialidase. Whether the observed enhancement of particulate sialidase activity correlates with the anesthetic potency of the substances studied, remains to be investigated.

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