CATECHOLAMINE SECRETION FROM THE ADRENAL GLAND TREATED WITH NEURAMINIDASE, PROTAMINE OR ALKYLATING AGENT

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It has been shown that brain gangliosides are concentrated in microsomal fractions and in synaptic membranes (1—3) and that the increased respiratory responses of cerebral cortex slices to stimulation are inhibited by basic proteins such as histones and protamines and alkylating agents which form covalent linkages in aqueous solutions with carboxylic and other acidic groupings (4, 5). Inhibition of the responses can be restored by polyacidic substances such as ganglioside and phosphatidylserine (4). On the other hand a possible role of ganglioside in release of acetylcholine from synaptic vesicles was also shown (6). It may be of interest to determine the intracellular distribution of gangliosides in the adrenal medulla as well as the possible effect of protamines, neuraminidase and nitrogen mustard on the adrenal medullary secretion.

Soon after death bovine adrenal glands obtained from the local slaughterhouse were placed on ice, then prepared for medullary cell-fractionation and perfusion. The medullae were homogenized in two volumes of 0.25 M sucrose. Each 0.8 ml of the homogenate was centrifuged in a 0.3–2.3 M sucrose gradient in the 5-ml Spinco preparative ultracentrifuge tubes at $125,000 \times g$ for 30 min according to the method of Potter and Axelrod (7). The subcellular fractions were dialyzed for 18 hr in running tap water, dried in vacuum, and suspended in a homogenizer in 100 ml of chloroform-methanol (2:1, v/v). The ganglioside fraction was extracted and estimated as described by Burton (8). Succinic dehydrogenase activity was estimated by the method of Slater and Bonner (9). Catecholamines (CA) in the subcellular fractions and the perfusate were assayed by the ethylenediamine condensation method (10). Method of perfusion of bovine adrenal glands was similar to that described by Trifaró et al. (11). CA secretion was evoked by switching perfusion from Locke's solution to Locke's containing 5×10^{-3} M of acetylcholine (ACh), or from Ca-free Locke's solution to Locke's, using perfusion pump. Flow rate was 15 ml/min. For the determination of the effect of neuraminidase, protamine or nitrogen mustard on the induced CA secretions, the following calculation was used.

$$\text{Percent of initial secretion} = \frac{st_2 - sp_2}{st_1 - sp_2} \times 100,$$

where sp_1 and st_1 were initial spontaneous release during incubation and the initial stimulated release of CA, respectively, sp_2 and st_2 were spontaneous and stimulated release of CA

TABLE 1. Subcellular distribution of gangliosides in bovine adrenal medulla.

Tissue fraction	Gangliosides	Catecholamines	Succinic dehydrogenase
Whole homogenate	28.7 (μ g, NANA)	100 (%)	2.81 (Δ O.D./min/g)
Supernatant	2.2	34.3	0.04
Microsomes	8.6	4.5	0.04
Mitochondria	1.8	10.0	1.18
Chromaffin granules	7.2	29.4	0.22

Values of ganglioside content and succinic dehydrogenase activity per gram of fresh adrenal medulla.

TABLE 2. Effect of neuraminidase, protamine and nitrogenmustard on acetylcholine- or calcium-induced catecholamine secretion from bovine adrenal gland.

Treatment	ACh-induced CA secretion		Ca-induced CA secretion	
	Per cent of initial secretion*	SP ₂ /SP ₁	Per cent of initial secretion**	SP ₂ /SP ₁
None	98.5 $\pm$ 8.7	1.1 $\pm$ 0.1	112.5 $\pm$ 10.3	0.9 $\pm$ 0.0
Neuraminidase, 0.24 U/ml, 30 min	<u>154.7</u> $\pm$ 6.7	1.7 $\pm$ 0.2	108.7 $\pm$ 9.3	1.0 $\pm$ 1.0
Protamine, 0.07 mg/ml, 5 min	<u>155.2</u> $\pm$ 10.4	1.7 $\pm$ 0.2	<u>87.6</u> $\pm$ 13.2	1.0 $\pm$ 1.1
Nitrogen mustard, 4.3 mM, 15 min	<u>171.5</u> $\pm$ 27.9	1.6 $\pm$ 0.3	93.6 $\pm$ 9.6	0.9 $\pm$ 0.1

Values are means and standard errors of 4 to 5 experiments. Underlined values were different from control value at a $p < 0.05$ by *t* o-sample *t*-test.

* Average st₁ and sp₁ were 16.3 and 3.4 (μ g/min), respectively. ** Average st₁ and sp₁ were 5.4 and 3.7 (μ g/min), respectively.

30 min after the initial stimulated secretion, respectively. Test compounds or enzyme were perfused between the initial and the second stimulations. The duration of perfusion and the concentration of the test compounds or enzyme are indicated in the Table 2.

Gangliosides were found in greater concentration in the microsomal fraction (29.9 per cent) and in chromaffin granules (25.0 per cent). CA, used as a marker of chromaffin granules, was the greatest concentration in chromaffin granule except supernatant. Succinic dehydrogenase activity, as a mitochondrial marker, was concentrated in the mitochondrial fraction (Table 1).

ACh (5×10^{-5} M) produced a 4.8 (average)-fold stimulation of CA secretion. Ca-reintroduction produced a 1.5 (average)-fold stimulation. ACh induced CA secretion was potentiated by the treatments of adrenal gland with neuraminidase, protamine or nitrogen mustard under such conditions as indicated in Table 2. Spontaneous CA secretions during incubation were found to be somewhat increased after treatments with the enzyme or the compounds (Table 2). Treatment with a high concentration of protamine (more than 0.2 mg/ml) or nitrogen mustard (more than 15 mM), however, caused increases in sp₂/sp₁ (not illustrated). On the contrary CA induced Ca secretion was not increased by treatment with neuraminidase, protamine or nitrogen mustard under such conditions as indicated in Table 2.

Treatment with protamine caused a decrease in per cent of initial secretion.

The above experiments were done to determine whether acidic components which may be involved in ACh receptor of chromaffin cells might be disturbed by neuraminidase, protamine or nitrogen mustard. Under the conditions tested in the present experiments, however, ACh induced CA secretion was potentiated by the enzyme or the compounds which may effect the acidic components of the cells. Ca induced CA secretion was not influenced by the enzymes or the compounds. When Ca is reintroduced it enters the cell and activates the amine extrusion mechanism of intracellular site (12), therefore it appears that neuraminidase or nitrogen mustard do not significantly interfere with the intracellular amine extrusion mechanism. Acidic components such as gangliosides which may be involved in chromaffin cell membrane may not be an essential factor in so-called stimulus-secretion coupling. It is of interest that ACh induced CA secretion was rather enhanced by the substances which effect acidic components.

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