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CONTRACTILITY OF ISOLATED AORTIC STRIPS FROM RATS WITH STABLE ARTERIAL HYPERTENSION DUE TO LONG-TERM ADMINISTRATION OF CEREBROSIDES

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There is growing factual evidence that neurochemical mechanisms participate in the regulation of the cerebral circulation [1, 2]. Further research in this direction has shown that glycosphingolipids (GSL), one of the neurochemical components of the brain, have a marked action on cerebral vessels [3] and reveal the cell-membrane mechanisms of their vasocontrictor effect [4]. These findings, and also data showing a raised blood GSL level in cerebrovascular pathology [5, 7] and arterial hypertension [11], and the ability of cerebrosides, under experimental conditions, to induce stable arterial hypertension [12], determined the aim of the present investigation, which was to study contractility of smooth-muscle cells (SMC) of strips of the abdominal aorta of rats during long-term administration of cerebrosides to the animals.

EXPERIMENTAL METHOD

Experiments were carried out on nonbred male albino rats weighing 140-160 g. The total cerebroside fraction was isolated from bovine brain [13] and then purified on a column with mark L silica-gel (Chemapol, Czechoslovakia). Cerebrosides in a dose of 5 mg/kg, in the form of a suspension made up in a mixture of ethanol and physiological saline (1:20), were injected intraperitoneally (0.5 ml) daily for 4 months. Animals of the control group received the same volume of the ethanol–physiological saline mixture. The blood pressure (BP) was measured by a noninvasive physiological saline mixture. The blood pressure (BP) was measured by a noninvasive

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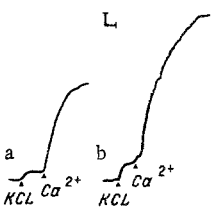

Fig. 1

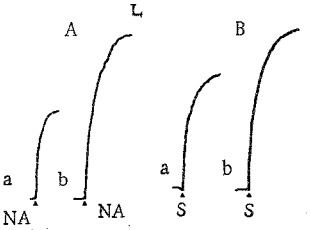

Fig. 2

Fig. 1. Contractile responses of depolarized (50 mM KCl) spiral strips of abdominal aorta of non-inbred albino rats to addition of 2.5 mM $CaCl_2$ to medium. Here and in Fig. 2, calibration: abscissa, time (2 min); ordinate, magnitude of contraction (80 mg); a) normotensive, b) hypertensive animals.

Fig. 2. Contractile response of spiral strips of abdominal aorta to noradrenalin – NA (10^{-7} M) and serotonin – S (10^{-7} M).

method on an instrument from "Ugo Basile" (Italy). The animals were decapitated, the abdominal aorta was removed, and spiral strips (2 × 20 mm) were cut from sections of it and placed in a constant-temperature chamber containing Krebs–Henseleit solution. The solution was constantly saturated with carbogen (95% O_2 + 5% CO_2); the pH and temperature were kept at 7.4 ± 0.05 and 37 ± 0.5°C respectively. The strips of aorta were adapted to the working chamber when stretched by a load of 1000 mg until the contractions became stabilized. The interval between additions of the test compounds to the medium was 20 min. Isometric contractions were recorded on an "Isotonische Messeinrichtung" instrument (West Germany). The results were subjected to statistical analysis by Student's test.

EXPERIMENTAL RESULTS

Chronic administration of total fraction of brain cerebrosides to the experimental animals led to a stable rise of BP. For instance, whereas in rats of the control group BP was 109 ± 3.8 mm Hg, in animals receiving cerebrosides it reached 138 ± 3.0 mm Hg ($P < 0.01$).

According to existing views [10], increased vascular tone in various forms of arterial hypertension is based on processes such as changes in permeability of the plasma membranes of SMC to monovalent ions, intensification of Ca^{++} inflow from the extracellular space, and disturbance of its binding with membranes of subcellular structures. Ultimately the accessibility of calcium for contractile proteins and the contractile activity of the vascular SMC are increased.

Accordingly, to study the functional state of the slow potential-dependent calcium channels (SPDCC), which play an important role in the maintenance of basal tone of SMC of arteries [14], potassium depolarization of SMC membranes (50 mM KCl) was induced in calcium-free Krebs–Henseleit solution, and this was followed by the addition of 2.5 mM $CaCl_2$. The fact will be noted that the contractile effect of $CaCl_2$ on contractile function of the arterial strips form rats receiving cerebrosides was much stronger (by 49%; $P < 0.05$) than in the control animals (Fig. 1a, b). This fact is evidence that activation of SPDCC in SMC of arteries of hypertensive rats, under conditions of potassium depolarization, takes place to a greater degree than in the control. The use of a hyperpotassium calcium-free Krebs–Henseleit solution containing manganese ions (10 mM), which block the permeability of calcium channels of plasma membranes and prevent release of intracellular Ca^{++} [9], showed that the potassium contracture developing under these conditions in strips of aorta from hypertensive and normotensive animals was almost equal in intensity. These results show, on the one hand, that there are no significant differences in the size of the intracellular Ca^{++} pool of SMC of arteries of control rats and rats receiving cerebrosides and, on the other hand, that the inflow of Ca^{++} into SMC of arteries of hypertensive animals along electrogenic channels is increased. This hypothesis is supported by the fact that GSL can be incorporated into plasma membranes, where they modify activity of Na^+,K^+-ATPase [6], which, in the plasma membranes of SMC, regulates the Ca^{++} level in the intracellular formation and, consequently, regulates processes of muscular contraction in the vessel wall [15].

To study the functional state of chemosensitive (receptor) calcium channels of arterial SMC the response of the aortic strips to addition of noradrenalin (10^{-7} M) and serotonin (10^{-7} M) to calcium-containing Krebs–Henseleit solution was studied. The experiments showed that the contractile effect of these vasoconstrictors on the arterial segment of animals with stable arterial hypertension, induced by long-term administration of cerebrosides, was much greater than in the control (Fig. 2a, b). When noradrenalin was used, it exceeded the control values by 44% ($P < 0.001$), but when serotonin was used, the increase was 29% ($P < 0.05$). Considering the results of our previous investigations concerning the ability of GSL to be inserted with the ceramide part of its molecule into model and biological membranes, and to induce conformational changes in them [8], it can be tentatively suggested that the increase in sensitivity of SMC of the aortic segments of hypertensive rats to noradrenalin and serotonin was due to structural and functional changes in the corresponding receptor formations or their microenvironment, extending also to Na^+,K^+-ATPase, arising after administration of cerebrosides.

The investigation thus showed that arterial SMC of rats with stable arterial hypertension, caused by long-term administration of cerebrosides, are characterized by increased inflow of extracellular Ca^{++} along electrogenic SPDCC and by hypersensitivity to noradrenalin and serotonin.

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