EFFECT OF VASOPRESSIN ON ELECTRICAL AND CONTRACTILE RESPONSES OF VASCULAR SMOOTH MUSCLES IN ANIMALS OF DIFFERENT AGES

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Most studies of the role of vasopressin in the regulation of cardiovascular function and the development of cardiovascular pathology have been devoted to an examination of changes in the central and regional hemodynamics [5, 6, 11]. Direct effects of the hormone on the vascular wall have been studied frankly insufficiently. The same can also be said of investigations into the effects of vasopressin at different ages. We know that systemic hemodynamic responses to vasopressin are modified in old age [3, 4]. However, changes in the functional state of vascular smooth-muscle cells (SMC) in old age have been inadequately studied.

The aim of this investigation was to analyze the effects of vasopressin on electrical and contractile properties of SMC of the femoral artery of adult and old rats and the possible role of cyclic AMP (cAMP) in its realization.

EXPERIMENTAL METHOD

Experiments were carried out on isolated spiral strips of the femoral arteries of adult (6-8 months) and old (24-26 months) rats. The preparations were perfused with Krebs' solution at 37°C. The membrane potential (MP) was measured by means of intracellular microelectrodes. A preliminary load of 600-800 mg was attached to the preparation. Contractile responses were recorded by means of a mechanical to electrical transducer. Lysine-vasopressin (from Serva, West Germany) in concentrations of $5 \cdot 10^{-8}$ to $5 \cdot 10^{-3}$ U/ml was used. Vasopressin was applied after the tone of the preparations had stabilized. Changes of tone were calculated per milligram wet weight of the strips. The cAMP concentration was determined under standard conditions and after incubation of the preparations in Krebs' solution containing $5 \cdot 10^{-4}$ U/ml of vasopressin, with the aid of a radioactive kit (Amersham International, England). cAMP was extracted from the tissues by the method in [12]. The calculations were done with the aid of standard curves; radioactivity was counted on a liquid scintillator (Chicago Nuclear, USA).

EXPERIMENTAL RESULTS

Perfusion of the muscle strips from the femoral artery of adult and old rats with solution containing vasopressin caused membrane depolarization of SMC and tonic contraction of the strips. Original curves obtained by recording electrical and contractile response of SMC from the femoral artery of adult and old rats to vasopressin in a concentration of $5 \cdot 10^{-3}$ U/ml are shown in Fig. 1. Exposure to this concentration of vasopressin caused considerable changes in membrane potential of SMC and in the level of tonic contraction. As Fig. 1 shows, depolarization of SMC and tonic contraction of the strip were more marked in adult than in old rats. This same relationship also was observed under the influence of lower concentrations of the hormone. Golovchenko [1] also found a greater increase in perfusion pressure in the coronary system of old animals compared with that of adult rats under the influence of the same vasopressin concentrations. The threshold vasopressin concentration evoking the first recordable changes in MP and tonic contraction of SMC in adult animals was $5 \cdot 10^{-6}$ U/ml. For SMC of the femoral artery of old animals the threshold concentration was an order of magnitude lower, namely $5 \cdot 10^{-7}$ U/ml, i.e., the threshold sensitivity of SMC to vasopressin is higher in old

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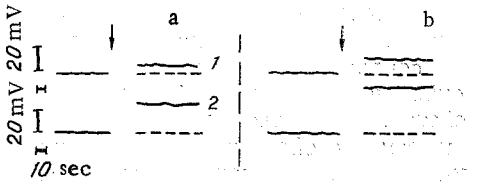

Fig. 1. Electrical (1) and contractile (2) responses of smooth muscles of femoral artery of adult (a) and old (b) rats to vasopressin (arrow) in a concentration of $5 \cdot 10^{-3}$ U/ml.

than in adult animals. A similar increase of sensitivity to vasopressin was demonstrated previously when its effect was studied on SMC of the portal vein of adult and old rats [2, 9]. Cumulative curves of the degree of depolarization and tonic contraction of SMC as functions of vasopressin concentration in the perfusion fluid, given in Fig. 2, show that curves obtained from old animals are shifted to the left, i.e., the sensitivity of SMC of the femoral artery of these animals to vasopressin was increased, and the angle of slope of the curves was steeper, i.e., the electrical and contractile responses of SMC of the old animals to vasopressin were more marked than in adult animals.

A feature of the action of vasopressin on the cardiovascular system is tachyphylaxis: in response to repeated administration of the hormone its hemodynamic effect decreased sharply. In the present investigation the development of tachyphylaxis was studied in isolated preparations of the femoral artery of adult and old animals. To reproduce electrical and contractile responses of SMC of adult animals of equal amplitude to vasopressin, the rinsing interval had to be not less than 35-40 min. If this interval was shortened, each successive response was weaker than its predecessor. When the intervals between the end of the previous application and the beginning of the next did not exceed 3-5 min, depolarization and tonic contraction of SMC of adult animals was not observed after three or four repeated applications (Fig. 3b). During frequent repetition of vasopressin application with a short rinsing period an opposite response to the hormone appeared: hyperpolarization and relaxation of preparations from the test vessels developed (Fig. 3c). Tachyphylaxis was much weaker in SMC of the femoral artery of old animals: repeated applications of vasopressin with a short rinsing period reduced the amplitude of the electrical and contractile responses only very slightly, but disappearance of the responses of SMC to the hormone was not observed in old animals, not did hyperpolarization and relaxation develop. For example, in SMC of adult animals five repeated applications of vasopressin in a concentration of $5 \cdot 10^{-3}$ U/ml with rinsing periods of 5 min led to hyperpolarization of SMC (6.5 ± 0.7 mV, n = 10) and to the development of relaxation (10 ± 3.6 mN, n = 10). Meanwhile SMC of old animals, under the same experimental conditions, responded with the development of depolarization (9.3 ± 2.1 mV) and tonic contraction (27.5 ± 5.6 mN; n = 12 for both parameters).

Responses of SMC of adult and old animals under the conditions described above were thus opposite in direction, and quantitative differences between them were statistically significant ($P < 0.01$). It can be tentatively suggested that the cause of the development of tachyphylaxis was the formation of a complex by the hormone molecule and the receptor, with the development of reversible receptor blockade. In a study of the effect of vasopressin on SMC of the portal vein of adult and old animals, it was shown that it may have both an excitatory (strengthening spontaneous electrical and contractile activity) and an inhibitory (inhibiting spontaneous activity) action [2, 7]. It has been postulated that there are two types of vasopressin receptors in vascular SMC: V_1 (excitatory) and V_2 (inhibitory) [2, 8]. Investigation of tachyphylaxis in the portal vein of adult animals showed that it effects only excitatory responses to vasopressin and the inhibitory action of the hormone was reproduced unchanged after repeated applications. In SMC of the femoral artery excitatory responses were observed in all preparations tested and with all concentrations of vasopressin used. It can accordingly be postulated that most receptors in vessels of this type were of the V_1 type. However, experiments to study the development of tachyphylaxis in SMC of the femoral artery of adult animals showed that during repeated application of vasopressin with a short rinsing period, an inhibitory action of the hormone could be detected. Inhibitory responses to vasopressin in this case are probably connected with the development of reversible blockade of V_1 receptors and manifestation of the action of the hormone on V_2 receptors. The number of inhibitory

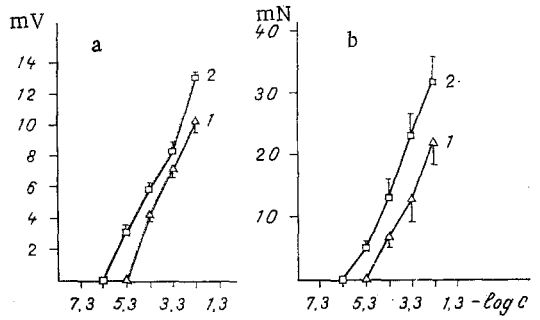

Fig. 2

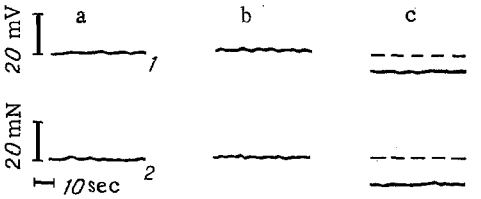

Fig. 3

Fig. 2. Degree of depolarization (a) and of tonic contraction (b) of smooth muscles of femoral artery of adult (1) and old (2) rats as functions of vasopressin concentration in perfusion fluid.

Fig. 3. Development of tachyphylaxis to action of vasopressin in a concentration of $5 \cdot 10^{-3}$ U/ml in smooth muscles of femoral artery of adult animals. a) Initial level of MP (1) and tonic contraction (2): b) after four repeated doses of vasopressin with a rinsing period of 5 min; c) after fifth application.

receptors was evidently sharply reduced in SMC of the old animals, or they were absent altogether. Adopting the hypothesis that there are two types of vasopressin receptors in vascular SMC and that V_1 (excitatory) receptors predominate in old animals, it can be postulated that insufficient protein synthesis of V_2 (inhibitory) receptors or excessive synthesis of V_1 receptors is one possible cause of the rise of the systemic blood pressure in old age.

A leading role in the mechanism of action of many hormones on cells is nowadays ascribed to cyclic nucleotides. The relaxing action of cAMP on contractile responses of isolated preparations of the aorta to vasopressin has been demonstrated [10]. However, the authors cited do not rule out the possibility that this action of cAMP may be unrelated to the mechanism of action of vasopressin on vascular SMC. Under the influence of vasopressin no change in the cAMP concentration was found in SMC of the portal vein of adults and old animals [2]. In the present experiments the effect of incubation of SMC preparations from the femoral artery of adult and old rats was investigated in a solution containing vasopressin in a concentration of $5 \cdot 10^{-4}$ U/ml, which evokes marked electrical and contractile responses. The cAMP concentration in the wall of the femoral artery has been shown not to change with age: it is 245 ± 29.1 pmoles/g in adult animals and 262 ± 26 pmoles/g in old animals. After incubation of vascular preparations for 5 min in a solution containing vasopressin, the cAMP concentration did not change significantly, but was 227 ± 48.3 and 229 ± 26.7 pmoles/g in adult and old animals, respectively. The action of vasopressin on vascular SMC is evidently not realized through a change in the intracellular cAMP concentration, and age differences in the responses of the vessels studied to vasopressin likewise do not depend on the cAMP concentration.

An increase in sensitivity to vasopressin is thus observed in vascular SMC of old animals and the intensity of responses to vasopressin in the concentrations used increases. Tachyphylaxis is much weaker in vascular SMC of old animals than of adult animals.

LITERATURE CITED

1. S. F. Golovchenko, Byull. Éksp. Biol. Med., 86, 647 (1976).
2. M. I. Gurevich and I. V. Frol'kis, Fiziol. Zh. (Kiev), 30, 107 (1984).
3. V. V. Frol'kis, S. F. Golovchenko, L. N. Bogatskaya, et al., Kardiologiya, No. 12, 103 (1976).
4. B. V. Pugach, Fiziol. Zh. (Kiev), 16, 823 (1970).
5. A. W. Cowley, E. W. Quillen, and M. M. Skelton, Fed. Proc., 42, 3170 (1983).
6. B. F. Ericsson, Acta Chir. Scand., 137, 414 (1971).
7. K. Golenhofen and A. N. Weston, J. Physiol. (London), 246, 55 (1975).
8. M. I. Gurevich and I. V. Frol'kis (Frolkis), Abstracts of the 10th European Congress of Clinical Gerontology, Budapest (1983), p. 65.
9. M. I. Gurevich and I. V. Frol'kis (Frolkis), Physiology of Cell Aging, Basel (1984), p. 111.
10. V. W. Kreye and C. Scimiz, Vasopressin. 2. Clinical Aspects, New York (1974), p. 90.

11. J. Nakano, Jpn. Circ. J., 37, 363 (1973).
12. A. L. Steiner, A. S. Pagliara, L. K. Chase, et al., J. Biol. Chem., 247, 1114 (1972).

BRAIN NORADRENALIN LEVELS IN RATS OF DIFFERENT AGES AFTER ADAPTATION TO A NEW ENVIRONMENT AND PASSIVE AVOIDANCE LEARNING

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KEY WORDS: noradrenalin; brain; stress; passive avoidance learning; ontogeny.

Significant changes in plasma 11-hydroxycorticosteroid (11-HCS) levels were found previously [2, 3] in rats of different ages after adaptation to a new environment and after passive avoidance learning (PAL).

Considering the close interaction between the pituitary-adrenal system and central adrenergic structures [4, 6], it was decided to study the effect of the above procedures on brain noradrenalin (NA) levels in rats of the same age groups.

EXPERIMENTAL METHOD

Male Wistar rats (90 aged 1 month, 88 aged 2 months) were used. Daily for 7 days the animals were placed for 3 min in a large illuminated compartment of a PAL apparatus, from which they spontaneously emerged through a round hole into a small, dark compartment (inborn preference for dark, small places). On the 8th day after the rats had remained for 3 min in the small compartment the hole was closed, and in the course of 1 min 20 bursts of ac pulses (50 Hz, 1 mA, duration of burst 0.5 sec) were applied to the grid floor. The presence of PAL (the animal did not move from the large into the small compartment during 3 min) was tested 24 h after electric foot shock (EFS). NA concentrations were determined fluorometrically by the trihydroxyindole method [5] in two parts of the brain: cortico-striatal (cerebral cortex, hippocampus, corpus striatum) and hypothalamus–brain stem (hypothalamus, thalamus, preoptic region, corpora quadrigemina). The brain was removed after decapitation of the animals in the animal house (basal NA level) or in the experimental room. The experimental results were subjected to statistical analysis by Student's test.

EXPERIMENTAL RESULTS

Comparison of the weight of the two brain regions studied in animals of both age groups showed that the hypothalamus–brain stem division of the brain develops much more rapidly than the cortico-striatal. The weight of the first brain division was 22.3% greater in rats aged 2 months than in those aged 1 month, whereas the weight of the second brain division was only 9.4% greater (285.0 ± 3.4 and 233.0 ± 2.1 mg, $P < 0.001$; 965.0 ± 8.5 and 882.0 ± 5.4 mg, $P < 0.001$, respectively). The rise in the basal NA level with age, on the other hand, was greatest in the unadapted animals in the cortico-striatal division (Fig. 1), in which it amounted to 65% ($P < 0.001$) compared with 34% ($P < 0.05$) in the hypothalamus–brain stem. Whereas in unadapted rats aged 1 month the NA concentration in the hypothalamus–brain stem division was almost 3 times higher than that in the cortico-striatal division, in animals aged 2 months it was only twice as high. Age changes in NA levels discovered in the different parts of the brain agreed with data in the literature [9, 12], according to which ontogenetic maturation of central noradrenergic pathways takes place in a strictly caudal-rostral direction, reflecting growth of axons and terminals from noradrenergic neurons from the brain stem.

Neither a new situation nor adaptation to it for 1 week (Fig. 1) led to any significant change in the NA level in the two brain divisions in rats aged either 1 month or 2 months. No changes likewise were found in the NA levels in animals of the two groups 15 min after EFS.

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