3. Yu. A. Vladimirov and G. E. Dobretsov, Fluorescent Probes in the Investigation of Biological Membranes [in Russian], Moscow (1980).
4. E. V. Gubler, Computer Methods of Analysis and Diagnosis of Pathological Processes [in Russian], Leningrad (1978).
5. S. V. Konev and I. D. Volotovskii, in: Biomembranes [in Russian], Riga (1977), pp. 42-77.
6. B. I. Kurganov, Allosteric Enzymes [in Russian], Moscow (1978).
7. N. M. Mirsalikhanova, E. E. Gussakovskii, S. Burkhanov, et al., Biofizika, 20, 980 (1975).
8. T. A. Odesskaya, Probl. Gematol., No. 2, 37 (1976).
9. I. S. Azhgikhina (editor), Prostaglandins [in Russian], Moscow (1978).
10. Z. A. Suslina, Zh. Nevropatol. Psikhiat., No. 11, 1635 (1978).
11. K. K. Turoverov and B. V. Shchelchkov, Boifizika, 15, 965 (1970).
12. A. D. Bangham, M. M. Standish, and J. C. Watkins, J. Mol. Biol., 13, 238 (1965).
13. J. George, R. Morgan, and P. Lawis, J. Lab. Clin. Med., 92, 430 (1978).
14. G. Dodge, C. Mitchell, and D. Hanahan, Arch. Biochem., 11, 100 (1963).
15. B. Samuelsson, M. Goldyre, E. Granström, et al., Annu. Rev. Biochem., 47, 997 (1978).
16. T. J. Wang, C. Hussey, E. Sasse, et al., Am. J. Clin. Pathol., 67, 528 (1977).
17. M. B. Feinstein, Bibl. Haemat., 45, 1 (1978).

MECHANISM OF THE RELAXING ACTION OF NORADRENALIN ON SMOOTH MUSCLE CELLS OF THE CORONARY ARTERIES

E. I. Nikitina, N. G. Kochemasova, V. M. Taranenko, and M. F. Shuba

UDC 612.181.612.172.1].014.46:615.357.452

KEY WORDS: smooth muscles; coronary arteries; basal tone; noradrenalin; calcium channels.

The basal tone of the coronary vessels and of the vessels of skeletal muscles, the mesentery, etc. is determined mainly by the inflow of external calcium ions into the smooth-muscle cells (SMC). Previously the writers showed that the inflow of calcium takes place through potential-dependent slow calcium channels [2, 11]. The aim of the present investigation was to continue the study of the membrane mechanisms of regulation of basal tone and, in particular, to examine the role of mediators (noradrenalin – NA) in these processes.

EXPERIMENTAL METHODS

Experiments were carried out on circular strips of the anterior descending branch of the bovine left coronary artery with an external diameter of 1.0-1.5 mm by the double sucrose gap method, with simultaneous recording of electrical and contractile activity of the SMC. Electrical activity was derived and the SMC stimulated by Ag–AgCl electrodes. Contact between muscle and electrodes was effected through agar bridges. The original Krebs' solution had the following composition (in mM): NaCl 120.4; KCl 5.9; $NaHCO_3$ 15.5; $MgCl_2$ 1.2; NaH_2PO_4 1.2; $CaCl_2$ 2.5; glucose 11.5 mM, made up in bidistilled water. Potassium-enriched solution (80 mM KCl) was prepared by adding the dry KCl salt to Krebs' solution; calcium-free solution was prepared by removing calcium ions from Krebs' solution by the addition of 0.5 mM EGTA. To stabilize the membrane, the $MgCl_2$ concentration in the calcium-free Krebs' solution was increased to 12 mM. NA was added in a concentration of 10^{-5} M and the testing solutions were aerated with a gas mixture containing 95% O_2 and 5% CO_2. The temperature of all solutions was maintained at 35-36°C. The preparations in the chamber were subjected to a load of 7×10^{-3} to 10×10^{-3} N. The experiments began 60-90 min after loading the preparations in the chamber.

Department of Neuromuscular Physiology, A. A. Bogomolets Institute of Physiology, Academy of Sciences of the Ukrainian SSR, Kiev. (Presented by Academician of the Academy of Medical Sciences of the USSR A. M. Chernukh.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 91, No. 5, pp. 517-520, May, 1981. Original article submitted July 17, 1980.

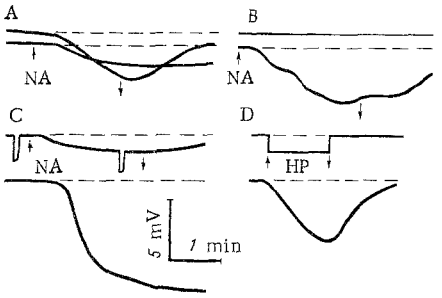

Fig. 1

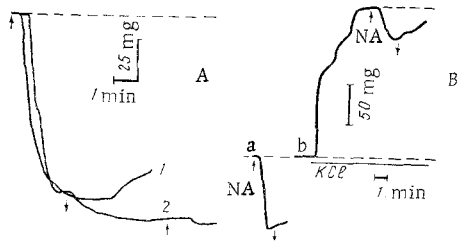

Fig. 2

Fig. 1. Action of NA and a polarizing current on electrical and contractile activity of SMC of the coronary arteries. Top traces show electrical, bottom traces contractile responses of SMC. Arrows on A, B, and C show beginning and end of action of NA, in D – beginning and end of action of hyperpolarizing current (HP).

Fig. 2. Action of NA on contractile activity of SMC of coronary arteries. A: 1) Action of NA in normal Krebs' solution, 2) action of calcium-free solution and beginning of action of NA against this background; B: a) action of NA in normal Krebs' solution; b) action of NA against the background of 80 mM KCl (continuous line). Arrows indicate beginning and end of action of substances.

EXPERIMENTAL RESULTS

The results showed that in approximately 50% of experiments NA induced hyperpolarization of the SMC membrane by 2-5 mV, and this was accompanied by relaxation of the muscle (Fig. 1A, B). Since hyperpolarization of the membrane in these experiments was connected with a decrease in its resistance (Fig. 1C), this could indicate that NA induces an increase in membrane conductance chiefly in relation to potassium ions. Other workers, studying the action of NA on the pig's coronary arteries [6], reached the same conclusion.

However, relaxation of SMC of the coronary arteries could also take place without any change in the resting potential (RP) level (Fig. 1B). It must be pointed out that Mekata and Niu [8], using a microelectrode technique, were unable in any of their experiments to observe a change in RP during relaxation of the coronary arteries of a dog under the influence of NA.

However, in those of the present experiments in which NA induced membrane hyperpolarization, the relaxing effect of NA could not be completely explained by the change in RP, for a change in RP to the same level by means of a hyperpolarizing current induced much less marked relaxation of SMC of the coronary arteries (Fig. 1D). Furthermore, on removal of the hyperpolarizing current, muscle tone was restored relatively quickly, whereas after discontinuation of the action of NA repolarization of the membrane never coincided with restoration of the tone of the muscle cells (Fig. 1A, C).

The writers' previous investigations showed that the inflow of calcium ions, regulating basal tone of the SMC of the coronary arteries, takes place through potential-dependent slow calcium channels [2, 11]. Accordingly, that part of the relaxation reaction to NA which is connected with hyperpolarization of the SMC membrane can be explained by closing of the potential-dependent slow calcium channels. However, this part of the reaction amounted to only 38-40% of the relaxation of the muscle cells under the influence of NA. How can the rest of the reaction (about 60%) be explained?

We know that a decrease in muscle tone may also be brought about through a reduction in the intracellular concentration of free calcium ions as a result of its binding in intracellular stores. The present investigations showed that after removal of calcium ions from the external solution NA was practically unable to produce further relaxation of the muscle cells (Fig. 2A). Relaxation under the influence of NA in calcium-free solution amounted to 7-12% of the reaction to NA in normal Krebs' solution. To explain the relaxing effect of NA which is not due to hyperpolarization and which does not involve binding of calcium in intracellular stores, it must be assumed that NA can inhibit the transmembrane inflow of calcium directly, through its action on potential-independent chemosensitive calcium channels. These hypothetical calcium channels in the usual state are open and, together with the potential-dependent slow calcium channels, they regulate the supply of calcium to the muscle cells, thereby maintaining the intracellular calcium concentration and the basal tone of the muscle at the necessary level.

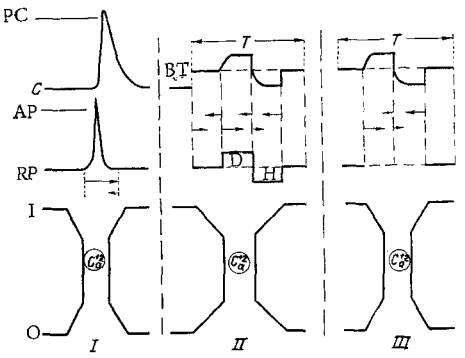

Fig. 3. Scheme of calcium channels of SMC membrane. I) Fast potential-dependent regenerative calcium channels, II) slow potential-dependent nonregenerative calcium channels, III) slow potential-independent chemosensitive calcium channels. Arrows to the right indicate number of open calcium channels, arrows to the left number of closed calcium channels. C) Contraction, PC) phasic contraction, T) tonic contraction, BT) basal tone, AP) action potential, RP) resting potential, D) depolarization, H) hyperpolarization, I) inner, O) outer side of membrane.

The existence of these channels is also confirmed by the fact that the reaction to noradrenalin is preserved in a muscle depolarized by potassium chloride. Under these conditions the chemosensitive potential-independent calcium channels remain open, whereas the effect connected with the action of NA on the potential-dependent calcium channels ought to be absent, for an increase in the potassium conductance of the membrane in cells depolarized by an increase in the external concentration of potassium ions ought not to be accompanied by hyperpolarization of the membrane. In fact, the relaxing action of NA on a muscle depolarized by potassium chloride was preserved (Fig. 2B, b) and amounted to 40-46% of the reaction to NA under normal conditions (Fig. 2B, a).

The β-effect of NA on the postsynaptic membrane of SMC of the coronary arteries can thus be represented as follows. On the one hand, NA causes an increase in potassium conductance of the membrane and, consequently, hyperpolarization of the membrane, and this leads to closing of the potential-dependent slow calcium channels and to a decrease in the transmembrane inflow of external calcium ions. On the other hand, NA acts on the open potential-independent chemosensitive calcium channels of the membrane and closes them, which also limits the inflow of external calcium ions into the muscle cells. As a result of this dual effect of NA the intracellular concentration of free calcium ions is considerably reduced and the basal tone falls.

In those cases when NA causes relaxation of the muscle without any accompanying change in RP, its effect is evidently due chiefly to closing of the chemosensitive potential-independent slow calcium channels of the membrane of SMC of the coronary arteries. This may take place when RP is close to the potassium equilibrium potential or when the chemoreceptors are mainly located on calcium channels.

The results of experiments to study the action of NA on SMC of the coronary arteries fill in some of the gaps in our ideas of the pathways of transmembrane inflow of external calcium ions into SMC. On the basis of the results of the writers' previous investigations obtained on SMC of the portal vein, ureter, and coronary arteries [1-5, 7, 9, 11], and also of experimental data described above, the presence of several types of calcium channels, through which external calcium ions enter the muscle cells and take part in maintenance of basal tone and active contraction, can be postulated in the membrane of SMC Fig. 3.

LITERATURE CITED

1. E. I. Nikitina, Fiziol. Zh. SSSR, No. 10, 1493 (1980).
2. V. M. Taranenko and N. G. Kochemasova, Byull. Éksp. Biol. Med., No. 10, 387 (1980).
3. V. M. Taranenko, N. G. Kochemasova, E. I. Nikitina, et al., Byull. Éksp. Biol. Med., No. 9, 311 (1978).

4. M. F. Shuba, V. M. Taranenko, and N. G. Kochemasova, Fiziol. Zh. SSSR, No. 8, 1200 (1980).
5. M. F. Shuba, V. M. Taranenko, and N. G. Kochemasova, Fiziol. Zh. SSSR, No. 8, 1209 (1980).
6. Y. Ito, K. Kitamura, and H. Kuriyama, J. Physiol. (London), 294, 595 (1979).
7. N. G. Kochemasova and M. F. Shuba, in: Physiology and Pharmacology of Smooth Muscle, Varna (1979), p. 118.
8. H. Mekata and H. Niu, Jpn. J. Physiol., 19, 599 (1969).
9. E. I. Nikitina, in: Physiology and Pharmacology of Smooth Muscle, Varna (1979), p. 92.
10. U. Peiper and E. Schmidt, Pflüg. Arch. ges. Physiol., 337, 107 (1972).
11. V. M. Taranenko and E. N. Kovaleva, in: Physiology and Pharmacology of Smooth Muscle, Varna (1979), p. 118.
12. E. Uchida and D. F. Bohr, Am. J. Physiol., 216, 1343 (1969).

USE OF SIMULTANEOUS RECORDING OF AUDITORY CORTICAL-EVOKED POTENTIALS AND COCHLEAR MICROPHONE POTENTIALS TO STUDY THE MECHANISM OF AUDITORY ADAPTATION

B. M. Sagalovich and G. G. Melkumova

UDC 612.858.7-08:[612.825.1+612.858.7].014.423

KEY WORDS: auditory adaptation; evoked potentials; cochlear microphone potentials

Intensive acoustic stimulation is known to reduce auditory sensitivity, i.e., to raise the thresholds of audibility. This rise of thresholds may persist for some time after the end of above-threshold acoustic stimulation. The amount of the rise in thresholds and the time required to restore their initial level are determined under normal conditions by the strength and duration of the acoustic stimulation. This fact is described in the literature under various names: "direct and reversed adaptation," "the fatiguing effect of noise," "the time shift of the auditory thresholds." However, ideas on the mechanism of this phenomenon are highly contradictory in character. Some workers ascribe the leading role to the central divisions of the auditory system [1, 6, 14], others to its peripheral formations and, in particular, the spiral organ [8, 11, 13] and, finally, others attach importance to the nonspecific systems of the brain stem [5, 7].

To shed light on this problem experiments were required with simultaneous recording of electrical auditory responses from central and peripheral structures of the auditory system, and the investigation described below was carried out for this purpose.

EXPERIMENTAL METHOD

Auditory evoked potentials (AEP) were recorded from the cortex and microphone potentials (MP) from the cochlea. The technique of deriving and recording the AEP was fully described by the writers previously [3, 4]. The animals were anesthetised with chloralose and pentobarbital. Cortical AEP was recorded in one channel of the recording system, MP from the cochlea were recorded synchronously in the other channel. MP was derived from the fenestra rotunda by means of a nichrome electrode. Approach to the cochlea was obtained through the bulla ossea. The electrode was fixed with acrylic glue.

Both potentials were reproduced by tonal stimulation with definite parameters. The duration of the tonal stimulus was 20 msec, the rise time 2.5 msec, and the filling frequency 0.5, 1.0, 4.0, and 8.0 kHz. Stimulating volleys were applied with a frequency of once per second. Stimuli were generated by an AUG-69 audiometer with an attachment enabling the assigned stimulus parameters to be formed. White noise was used as the above-threshold strong stimulus. The intensity of the noise was 90 dB above the 2×10^{-5} P level, and exposure lasted 10 min. The noise was generated by means of a second AUG-64 audiometer. The sound emitters

Laboratory of Pathophysiology, Moscow Research Institute for the Ear, Nose and Throat, Ministry of Health of the RSFSR. (Presented by Academician of the Academy of Medical Sciences of the USSR A. N. Chernukh.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 91, No. 5, pp. 520-522, May, 1981. Original article submitted July 18, 1980.

