previously on the adrenolytic properties of thioproperazine [8]. The β-blocking effect of trifluperidol, which we found, is evidence that the rat reticulocyte membrane preparation can be effectively used as a biochemical test system for estimating the effect of drugs on the β-adrenergic receptor coupled with AC. Affinity constants of thioproperazine for trifluperidol are 3 orders of magnitude lower than those of the known β-blockers propranolol and alprenolol [3], but the doses of these drugs used in clinical practice suggests that the adrenolytic action of trifluperidol and thioproperazine may play a significant role in the manifestation of their side effects.

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EFFECT OF NONACHLAZINE ON THE ADENYLATE CYCLASE SYSTEM OF THE RABBIT HEART

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KEY WORDS: adenylate cyclase; β-adrenoreceptors; myocardium; nonachlazine, ischemic heart disease

The drug nonachlazine — 10-β-[1,4-diazobicyclo-(4,3,0)-nonanyl-4-]-propionyl-2-chlorophenothiazine dihydrochloride — synthesized in the Institute of Pharmacology, Academy of Medical Sciences of the USSR, is used in the treatment of ischemic heart disease. The pharmacologic properties of nonachlazine have been investigated in detail [1]. The study of the effect of nonachlazine on cardiac activity (cardiac output, contractility) has shown that this compound possesses not only β-adrenostimulating properties, but also the ability to induce a partial β-adenoblockage [1]. We know that the β-adrenergic effect in animal cells is realized through the adenylate cyclase system (ACS) [3]. The molecular mechanisms of action of nonachlazine on adrenergic structures of the myocardium have not been elucidated.

The aim of this investigation was to study the effect of nonachlazine on the ACS of the rabbit heart, on regulation of adenylate cyclase (AC) activity by isoproterenol and also on binding of the β-adrenoreceptor antagonist [^{3}H]-dihydroalprenolol (DHA).

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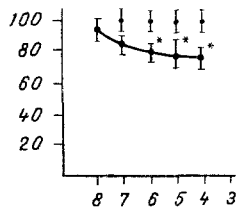

Fig. 1

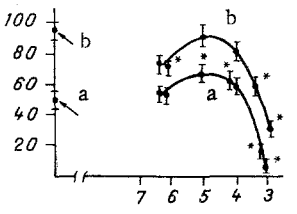

Fig. 2

Fig. 1. Effect of nonachlazine on specific binding (in %) of [^{3}H]-DHA by rabbit heart β-adrenoreceptors. Specific binding of [^{3}H]-DHA in absence of nonachlazine taken as 100%. Here and in Fig. 2, abscissa, negative logarithm of nonachlazine concentration (–log [M]). Here and in Figs. 2 and 3: *$p < 0.05$.

Fig. 2. Effect of nonachlazine on basal (a) and isoproterenol-stimulated (b) AC activity of rabbit heart. Ordinate, specific AC activity (in pmoles cAMP/mg protein/min). Isoproterenol concentration $5 \cdot 10^{-5}$ M.

EXPERIMENTAL METHOD

Rabbit heart membranes were isolated by the method in [2]. The membrane preparations were kept at the temperature of liquid nitrogen. AC activity was determined by the method in [6]. Material was incubated at 30°C in medium containing 50 mM Tris-HCl (pH 7.5), 5 mM $MgCl_2$, 0.5 mM cAMP, 0.5 mM isobutylmethylxanthine, 0.1 mM GTP, 20 mM creatine phosphate, 0.2 U creatine phosphokinase, 0.1 mM ATP, and 0.5 μCi (α-^{32}P)-ATP. The protein content in the sample was 10-30 μg. Binding of DHA with β-adrenoreceptor of rabbit heart membranes was measured by the method in [9]. The protein concentration was determined by the method in [11]. The following reagents were used: Tris, imidazole, cAMP, ATP, GTP, isoproterenol, EDTA, creatine phosphate, $MgCl_2$, isobutylmethylxanthine, and bovine serum albumin were from Sigma (USA), creatine phosphokinase from Boeringer (West Germany), and (α-^{32}P)-ATP and [^{3}H]-DHA from Amersham International (England).

EXPERIMENTAL RESULTS

The study of the effect of nonachlazine (10^{-8}-10^{-4} M) on binding of DHA with the β-adrenoreceptors of rabbit heart showed (Fig. 1) that the drug partially displaces the radioligand from the receptor (up to 20% in a concentration of 1 μM). Nonachlazine, like other phenothiazines, is known to have local anesthetic properties. However, displacement of DHA in micromolar concentrations is evidently not connected with the manifestation of these properties of nonachlazine, for local anesthetics displace DHA from β-adrenoreceptors with millimolar affinity [15].

The study of the effect of nonachlazine on AC activity showed that in micromolar concentrations the drug increases basal activity (Fig. 2a). With a further increase in the nonachlazine concentration AC activity fell considerably to not more than 20% of its initial value. In the membrane preparation which we used, isoproterenol in a saturating concentration of $5 \cdot 10^{-5}$ M stimulated AC activity by 50-100%. Nonachlazine ($5 \cdot 10^{-7}$-10^{-6} M) reduced the isoproterenol-stimulated AC activity by about 20% (Fig. 2b), a result which can evidently be explained by the β-blocking action of the drug. An increase in the nonachlazine concentration to 100 μm restored the original AC activity. With a further increase in the nonachlazine concentration, isoproterenol-stimulated activity fell sharply, in the same way as the basal activity.

Many compounds with local anesthetic properties, including several phenathiazines, modifying the physicochemical state of membranes [8, 10, 12, 13], exhibit a similar effect on basal and hormone-stimulated activity. The increase in basal activity in the presence of micromolar concentrations of nonachlazine can evidently be linked with facilitation of interaction between the components of ACS. However, in a concentration of 10^{-6} M, nonachlazine also had a partial β-blocking action, and for that reason the summation of the two effects caused virtually no change in isoproterenol-stimulated AC activity. In millimolar concentrations nonachlazine strongly inhibited AC activity (Fig. 2a, b), when it evidently exhibited a local anesthetic-like membrane-stabilizing action, as well as a direct effect on the functioning of components of the AC complex [15]. The membranotropic effects of local anesthetics are manifested by their ability to change the flowability of the cell membranes, and thereby to influence the number of collisions between components of the ACS (the "coupling or collision

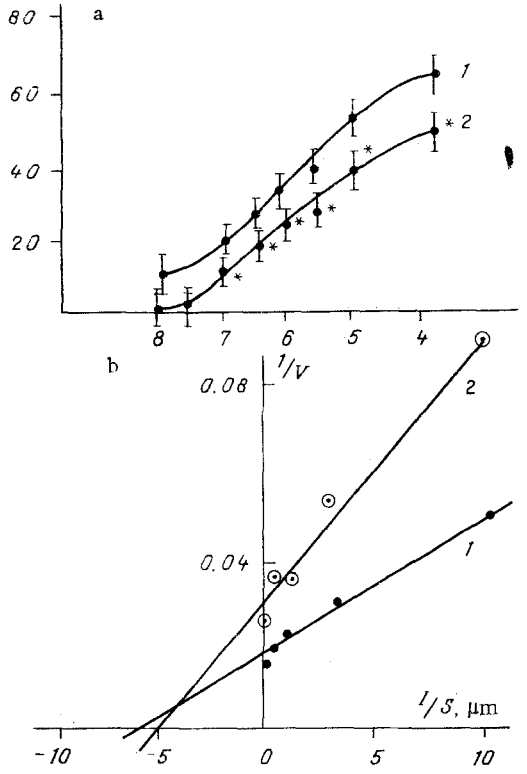

Fig. 3. Effect of nonachlazine on isoproterenol-stimulated AC activity of the rabbit heart between ordinary coordinates (a) and on Lineweaver–Burk plots (b). a: abscissa, negative logarithm of isoproterenol concentration (–log [M]); ordinate, stimulation of AC activity compared with basal value (in %); b: the same, on Lineweaver–Burk plot. 1) AC activity in presence of isoproterenol; 2) AC activity in presence of isoproterenol and nonachlazine ($5 \cdot 10^{-7}$ M).

hypothesis") [7, 10]. The direct action of these agents on regulation of ACS is expressed both by their ability to reduce affinity of the receptor for the hormone and to convert the receptor from a state of high affinity to low, and also by their property of influencing the regulatory effects of guanyl nucleotides, possibly through interaction with N-proteins [15].

To determine the character of inhibition of isoproterenol-stimulating AC activity, we studied the effect of nonachlazine on the relationship between activity of the enzyme and isoproterenol concentration. Isoproterenol stimulated basal AC activity with an activation constant of $2 \cdot 10^{-7}$ M (Fig. 3a). Nonachlazine ($5 \cdot 10^{-7}$ M) shifted the curve of dependence of AC stimulation by isoproterenol to the right, reducing specific activity on average by 20%. Analysis of the results on a Lineweaver–Burk plot showed that inhibition of isoproterenol-stimulated AC activity by nonachlazine within the concentration range from 10^{-7} to 10^{-5} M is virtually noncompetitive in character (Fig. 3b).

The results obtained are evidence that nonachlazine possess weak β-adrenoblocking properties, in agreement with results obtained previously in vivo [1]. For instance, inhibition of pulse responses to isoproterenol [1] may be the result of the β-blocking effect of the drug. Physiologically, the β_1-stimulating effect of nonachlazine was manifested by the development of a positive inotropic effect (strengthening of the contractile function of the heart, increased cardiac output). On the one hand, the positive inotropic action of the drug may be explained by its ability to inhibit noradrenalin reuptake [1]. On the other hand, the ability of nonachlazine to increase basal AC activity in micromolar concentrations which, if the high therapeutic doses are taken into account, may be present in the body, may lead to the same physiological consequences.

It could be postulated that the unequal action of nonachlazine on inotropic and chromotropic function reflects the presence of functionally different β-adrenoreceptors in the heart, performing chiefly inotropic or chronotropic functions. Pharmocologic proof of the existence of such types of receptors has been given in the literature [4, 5, 14]. These effects, according to data obtained by different workers, may be realized by either β_1- or β_2-types or by two subpopulations of β_1-adrenoreceptors [4, 5]. The negative chronotropic action of nonachlazine, and also its ability to induce some degree of hypertension, do not rule out its possession of β_2-blocking properties. However, our investigations showed that nonachlazine (10^{-8}–10^{-3} M) is unable to displace DHA from β_2-adrenoreceptors of rabbit lung membranes (data not given). Thus, the partial β-blocking action of nonachlazine is evidently realized through β_1-adrenoreceptors.

Our results may explain why nonachlazine possesses partial β-adrenoblocking properties together with its physiologically manifested β-stimulating activity. As was noted previously [1], this mechanism of self-limitation of its own sympathomimetic activity may perhaps play a role in the realization of some positive properties of nonachlazine and, in particular, its ability to potentiate the inotropic function of the heart without the development of tachycardia or an increase in the oxygen consumption of the heart.

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EFFECT OF MORPHINE ON SUPRASEGMENTAL AND SEGMENTAL MECHANISMS OF BLOOD PRESSURE REGULATION DURING PAIN

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KEY WORDS: sympathetic activity; blood pressure; nociceptive responses; morphine; segmental mechanisms

Morphine-like compounds, which effectively inhibit emotional and motor manifestations of pain, have virtually no effect on nociceptive hemodynamic responses [2, 7, 9]. It has been suggested that the uncoupling of these effects is based on dissociation in the action of opioids on modulating influences of the analgesic systems of the brain relative to emotions and autonomic activities, and increased resistance to analgesics of neuronal structures in the spinal cord – the final component of vasomotor regulation [3]. The resistance of nociceptive responses of the circulation may also be due to the central activating action of morphine on the sympathetic system, discovered by the writers previously [1]. However, the neurophysiological processes and brain levels responsible for the formation of this effect of morphine have not been investigated.

For the reason given above, it was decided to study the role of the segmental and suprasegmental structures in the realization of the activating effect of morphine on sympathetic mechanisms of vasomotor regulation.

EXPERIMENTAL METHOD

Unanesthetized curarized cats with an intact brain (30 animals) and with the spinal cord divided at the T7-T8 level (five animals) were used. The common peroneal and splanchnic

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