PHYSIOLOGY

Endogenous β-Adrenoreceptor Blocker

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Certain dilutions of human and animal serum and human liquor, urine, saliva, and amniotic fluid produce β-adrenoblocking effect, i.e., decrease the ability of epinephrine to inhibit spontaneous contractions of rat uterine myometrium. It is concluded that biological fluids contain endogenous β-adrenoblocker.

Key Words: *β-adrenoreactivity; adrenomodulators*

Based on the finding that the extract from the myometrium of pregnant cats reduces β-adrenoreactivity of the uterine muscle of nonpregnant rats, we have hypothesized that this effect is due to the presence of an endogenous β-adrenoblocker (EBAB) [1]. However, this hypothesis was not confirmed, although the evidence supporting it has been accumulated. It was demonstrated that protein kinase A, β-adrenoreceptor (BAR) kinase, and the intracellular factor β-arrestin are involved in BAR inactivation [5,10]; anti-BAR antibodies were detected in plasma of patients with bronchial asthma [12] as well as antibodies to myocardial BAR in rat blood [9], and a factor inhibiting cardiac response to isoprenalin in blood of pregnant rats [6]; a blocker of tracheal and bronchial myocyte BAR was identified in guinea pigs during anaphylaxis [8]. On the other hand, neurokinin Y, endothelin [11], lipid peroxidation products [3,12], β-phenylalanine [7], and other compounds reduce β-adrenoreactivity. The aim of the present study was to check up the hypothesis on the presence of EBAB in biological fluids.

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MATERIALS AND METHODS

The method is based on the ability of the test compound to decrease β-adrenoreactivity of strips cut from the uteral horn of a nonpregnant rat. This preparation is highly sensitive to epinephrine and other BAR agonists (inhibitors of spontaneous contractile activity, SCA). The possible effects of biological fluids on the preparation [2,4] and the stability of the epinephrine effect after repeated 10-min applications were taken into consideration.

Myometrial strips (8-10 mm long and 2-3 mm wide) were prepared during metestrus or diestrus and perfused with Krebs solution. Their contractions were recorded in a six-channel Miocytograph apparatus at 38°C and a perfusion rate of 0.7 ml/min. Epinephrine was added for 10 min before, during (11th-20th min), and after 20-min incubation with test fluid. Epinephrine was added in the concentration inducing pronounced inhibition of SCA of myometrial strips: $1\text{-}5\times10^{-10}$ or $1\text{-}5\times10^{-9}$ g/ml in cold season and two orders of magnitude higher in warm season. Biological fluids (serum of 6-year-old children, adult males, nonpregnant, pregnant, and delivering women, plasma, urine and saliva of pregnant women, amniotic fluid obtained from pregnant and delivering women, serum of newborns, liquor from males and

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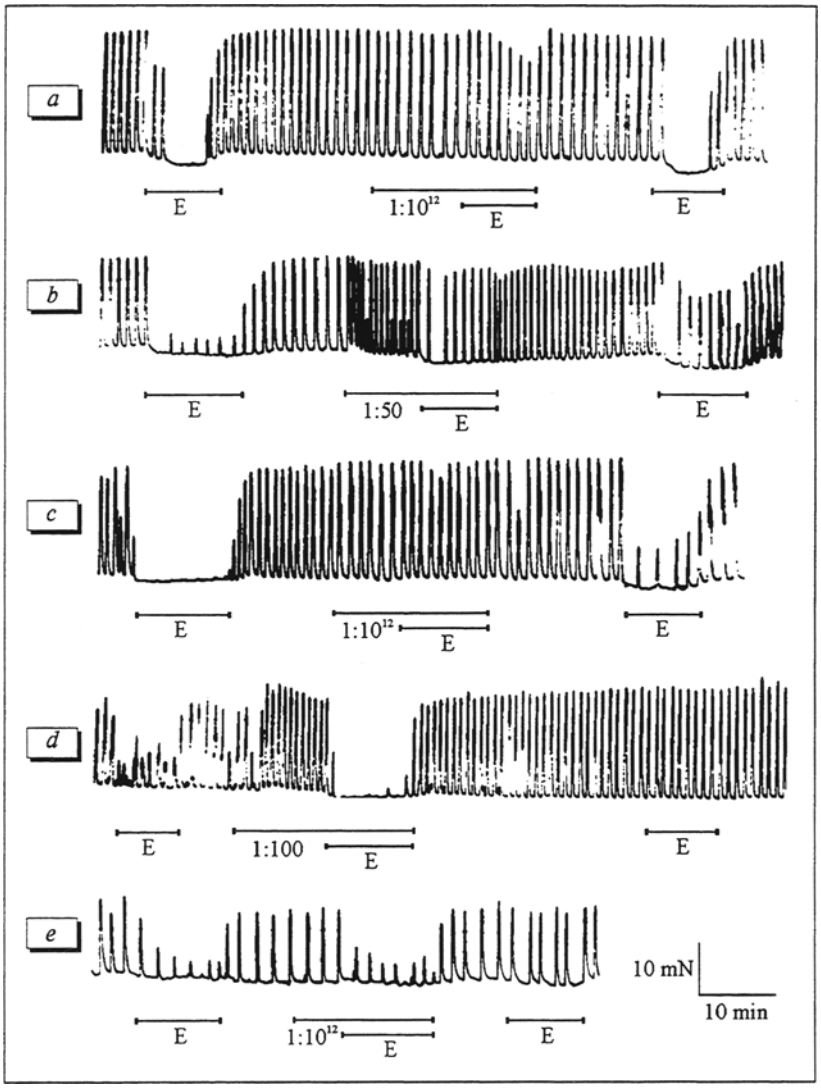

Fig. 1. Effects of human and animal sera on contractile activity and β-adrenoreactivity of longitudinal strips cut from the uterus of nonpregnant rats. Serum of man (*a*), bull (*b*), nonpregnant (slaughting) cow (*c*), nonpregnant woman (*d*), and pregnant cow (*e*). *E*) addition of 10^{-8} (*a*, *b*, *d*, *e*) or 10^{-9} g/ml epinephrine (*c*).

females, and serum of bulls, and pregnant and nonpregnant cows) were centrifuged for 15 min at 2000 or 3000 rpm and diluted 10-, 50-, 100-, 500-, 10^3-, 10^4-, 10^6-, 10^8-, 10^{10}, and 10^{-12}-fold with Krebs solution. Each dilution was analyzed for the presence of EBAB. The probability of β-adrenoblocking effect was estimated for each dilution, and the dilution—effect probability curve was constructed analogously to the dose—effect curve. A total of 350 human and animal biological fluids were tested on 2300 myometrium preparations of 370 rats.

RESULTS

All tested biological fluids increased (Fig. 1, *b*, *d*) or decreased (Fig. 1, *e* and Fig. 2, *b*, *d*, *e*) SCA of myometrial strips irrespective of the addition of epinephrine before or after the fluid. Generally, 10-, 50-, and 100-fold dilutions increased SCA, while higher dilutions (up to 10^8-10^{12}-fold) decreased it. Liquor, saliva, and amniotic fluids diluted 10-fold produced an inhibitory effect. At the same time, biological fluids modulated β-adrenoreactivity of the preparation (Fig. 1, *a-c*, Fig. 2, *a-e*), i.e., produced β-adrenoblocking effect manifesting itself as a decrease in the inhibiting activity of epinephrine on the SCA of myometrial strips. Blocking effect was often observed with 10, 10^6, 10^8, and 10^{10} dilutions (Fig. 3). Washing restored β-adrenoreactivity of the strips to practically initial level (Fig. 3); in some cases the restoration was fast (Fig. 1, *a*, *c*) and slow in others (Fig. 1, *b*, Fig. 2, *a-e*).

Two control series of experiments showed that repeated 10-min incubations of the strips with the same concentrations of epinephrine at 10-25-min intervals had no significant effect on β-adrenoreactivity. In the first series (n=12), the first addition of 10^{-7} g/ml epinephrine decreased the total contractible activity (the sum of amplitudes for a 10-min period) to 20±5.5% of the initial level, the second to 28±

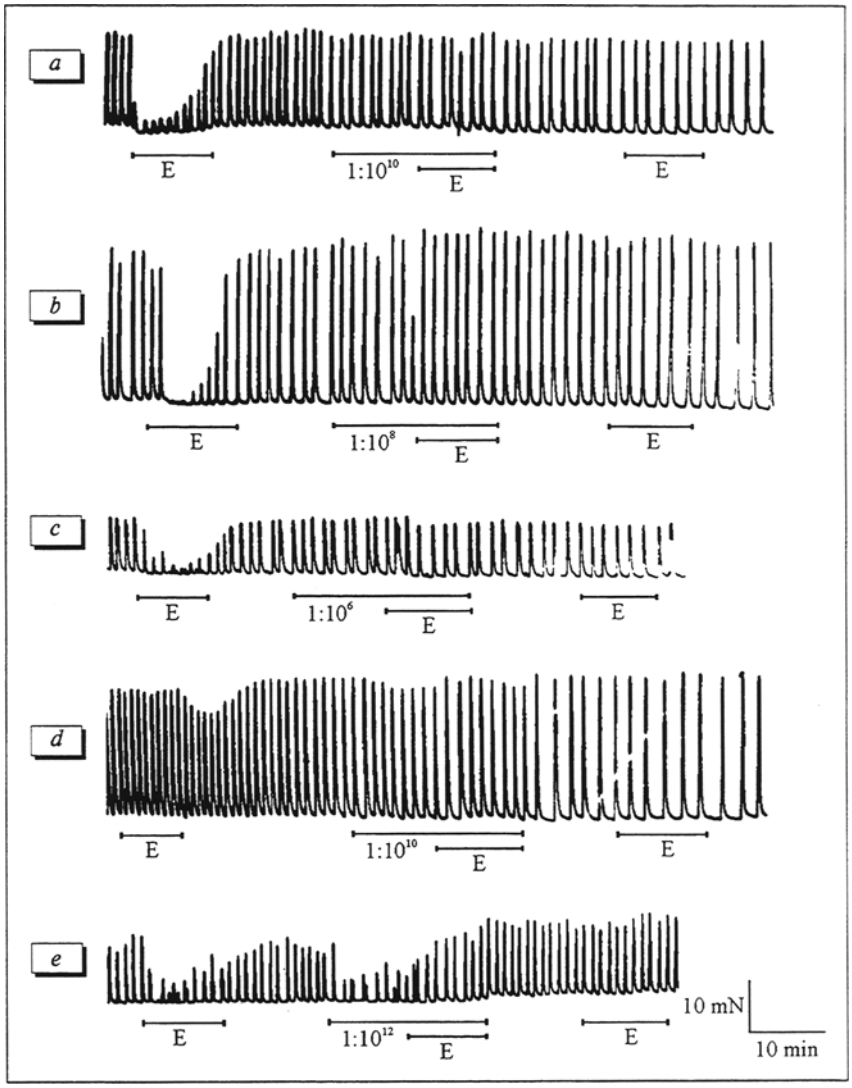

Fig. 2. Effect of human biological fluids on contractile activity and β-adrenoreactivity of longitudinal strips cut from the uterus of nonpregnant rats. Serum (*a*), urine (*b*), and saliva (*c*) of a pregnant woman (third trimester), amniotic fluid (*d*), serum prepared from umbilical blood of a newborn (*e*). *E*) addition of 10^{-8} g/ml epinephrine.

8.1%, and the third to 39±10.1% (the differences being insignificant, $p>0.5$). After the fourth addition, the inhibiting effect of epinephrine slightly decreased: the total activity dropped to 49±10.6%. In the second series ($n=21$), the response to epinephrin remained the same after the fourth addition of 10^{-11} or 10^{-7} g/ml epinephrine. Thus, in the absence of biological fluids β-adrenoreactivity of the strips is stable.

The reversible decrease in β-adrenoreactivity caused by biological fluids is due to the presence of EBAB in them. The nature of EBAB was not studied in this investigation. Judging from the probability of β-adrenoblocking effect of human serum (Fig. 3), there are two forms of EBAB: free and conjugated. At 10-fold dilutions, when the probability of the effect is maximal, free form predominates. At higher dilutions, the effect first declines and then increases (starting from 10,000-fold dilution). This may be due to a gradual increase in the amount of EBAB bound to its carriers and a decrease in the amount of free EBAB.

The content and the ratio between free and bound EBAB depend on the type of biological fluid (in pregnant women, the EBAB content of saliva is lower than that of serum and urine), sex (serum content of free EBAB is higher, while that of bound EBAB is lower in men than in nonpregnant women), and reproductive process (serum content of free EBAB in delivering women is higher that than in nonpregnant women).

The mechanograms shown in Fig. 1, *d*, *e* illustrate a decrease in β-adrenoreactivity of myometrial strips developing after removal of biological fluid (often after a β-sensitizing effect). We believe that this is a consequence of a pronounced desensitizing effect, i.e., activation of BAR phosphorylation by protein kinase A and BAR kinase of the uterine myometrium. Therefore, it is reasonable to distinguish between two β-adrenoblocking effects of a biological fluid: in the presence of the fluid and after "washing." The first effect probably reflects the

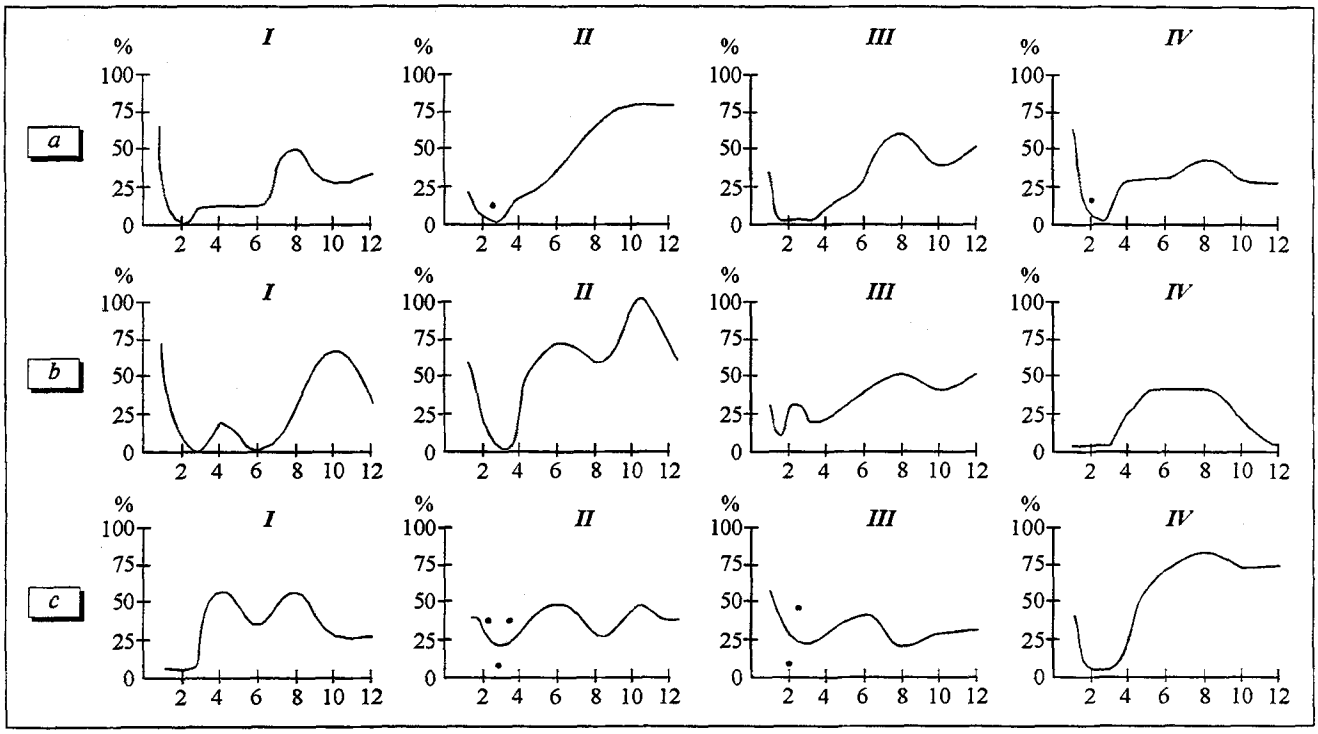

Fig. 3. Dilution—probability of β-adrenoblocking effect curves for longitudinal strips cut from the uterus of nonpregnant rats. *a*) human serum: *I*) men, *II*) nonpregnant women, *III*) pregnant women (28th-30th week), *IV*) delivering women. *b*) biological fluids of pregnant women (36th-39th week): *I*) serum, *II*) urine, *III*) saliva, *IV*) amniotic fluid obtained during delivery. *c*) animal sera: *I*) nonpregnant (slaughting) cows, *II*) nonpregnant cows, *III*) pregnant cows; *IV*) serum prepared from umbilical blood of human newborn. Abscissas: negative lg of dilution; ordinates: percent of experiments in which the effect was observed.

presence of EBAB in the medium, while the second effect is due to the presence of BAR-desensitizing factors.

Our results confirm the hypothesis that biological fluids contain endogenous b-adrenoblockers together with endogenous BAR-sensitizing factors, are involved in the mechanisms modulating β-adrenoreactivity of organs and tissues.

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