EFFECT OF ETHMOZINE DIETHYLAMINO ANALOG ON THE FORCE OF CONTRACTION AND ACTION POTENTIAL OF THE GUINEA PIG MYOCARDIUM

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KEY WORDS: ethmozine diethylamino analog; sodium-calcium exchange; force of contraction; slow response.

A new preparation of the phenothiazine series, namely ethmozine diethylamino analog (ethmozine DAA), which has a powerful antiarrhythmic action [4, 6-8], causes a decrease both in the inward fast sodium current [5, 6] and in the slow inward current [3, 6], in experiments on isolated fragments of myocardium.

Since a decrease in the inward current may affect the inotropic state of the heart muscle [1, 2, 12, 14], in the investigation described below the effect of ethmozine DAA on the force of contraction and the transmembrane action potential (AP) of papillary muscle was studied.

EXPERIMENTAL METHOD

A papillary muscle from the right ventricle of the guinea pig's heart was placed in a perfusion chamber (volume 0.5 ml, perfusion rate 10 ml/min) and perfused with Tyrode solution of the following composition (in mM): NaCl — 118.4, KCl — 2.7, $NaCHO_3$ — 25, NaH_2PO_4 — 1.2, $MgCl_2$ — 1.2, $CaCl_2$ — 1.8, glucose — 10; the solution was oxygenated with carbogen (95% O_2 + 5% CO_2) and its pH was 7.4 and temperature 36 ± 0.5°C. The preparation was stimulated with square electrical pulses 1 msec in duration and twice the threshold strength; the frequency of stimulation was 0.8 Hz. Flat Ag-AgCl electrodes, not touching the preparation, were used for stimulation. The force of contraction was measured by means of a 6MKh1S (USSR) mechanotron. Transmembrane AP were recorded with glass microelectrodes (3 MKCl). The maximal rate of rise of AP ($\dot{V}_{max}$) was obtained by means of a differential amplifier, linear up to 500 V/sec. When the slow calcium AP (Ca-AP) was investigated the sodium channels were inactivated by inceasing the K^+ concentration in the Tyrode solution from 2.7 to 18 mM without compensating osmolarity; under these circumstances the frequency of stimulation fell to 0.1 Hz and the Ca^{++} ion concentration in the Tyrode solution was doubled. All the parameters recorded were displayed on the screen of a "Tektronix-5115" oscilloscope (USA); the force of contraction was recorded continuously on a "Servogor" (USA) automatic writer. The duration of testing of one concentration of ethmozine DAA was usually 30-40 min, which was long enough for steady-state values of all the recorded parameters to be reached. The force of contraction and AP had not recovered 3 h after rinsing with Tyrode solution not containing the drug. Ethmozine DAA used in the experiments was synthesized in the Research Institute of Pharmacology, Academy of Medical Sciences of the USSR. The experimental results were subjected to statistical analysis by the paired t test and represented in the form M ± m.

EXPERIMENTAL RESULTS

The effect of ethmozine DAA on the force of contraction and on AP was tested over the concentration range from 1×10^{-7} to 1×10^{-5} g/ml. The typical action of ethmozine DAA in a concentration of 3×10^{-6} g/ml is demonstrated in Fig. 1. The principal electrophysiological manifestation of the action of ethmozine DAA was a decrease in $\dot{V}_{max}$. On the average in five experiments with such a high concentration of ethmozine DAA $\dot{V}_{max}$ fell to 53 ± 4% of the control, whereas the duration of AP fell to 97 ± 2% ($P > 0.05$) and the resting potential was reduced by 0.8 ± 1.2 mV. Inhibition of $\dot{V}_{max}$ developed monoexponentially (Fig. 2a, 1). The mean values of the time constants of inhibition of all parameters recorded were as follows:

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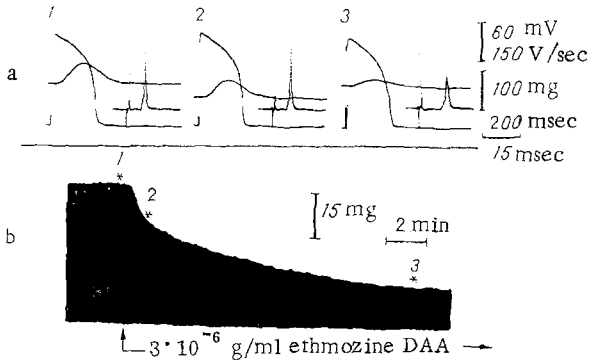

Fig. 1. Action of ethmozine DAA in a concentration of 3×10^{-6} g/ml on AP, $\dot{V}_{max}$, and force of contraction. a) Single cycles; b) continuous trace (numbers indicate times of recording AP and of single cycles of contraction).

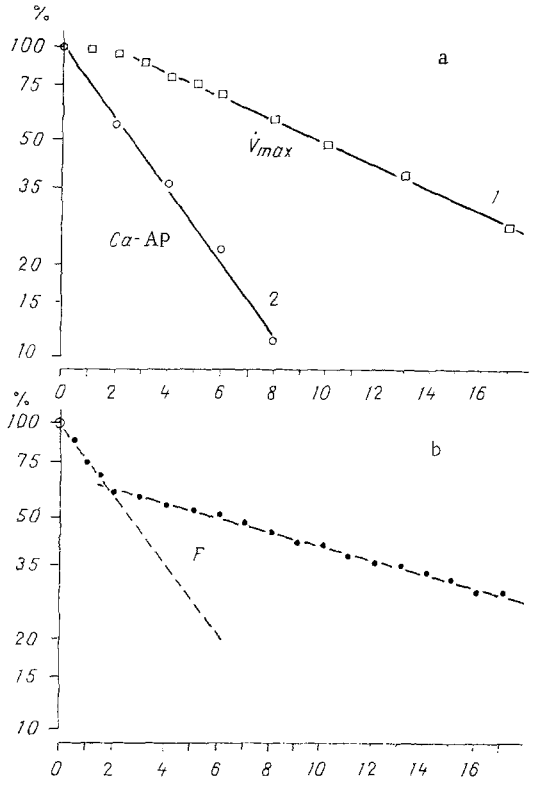

Fig. 2. Time course of development of action of ethmozine DAA in a concentration of 3×10^{-6} g/ml. a) Inhibition of $\dot{V}_{max}$ (1) whereas τ_V = 665 sec and overshoot of Ca-AP (2) with $\tau_{Ca\text{-}AP}$ = 228 sec; b) inhibition of force of contraction (experimental data are approximated by two exponents with τ_{F_1} = 233 sec and τ_{F_2} = 970 sec). Ordinate, value of $(y - y_\infty)/(y_0 - y_\infty) \cdot 100\%$ on logarithmic scale, in which y is the recorded value of the parameter (V_{max}, overshoot of Ca-AP, or force of contraction); y_0 is the value of this parameter in the control, y_∞ its value established under the influence of ethmozine DAA; abscissa, time from addition of ethmozine DAA to perfusate (in min).

$\tau_{Ca\text{-}AP}$ = 220 ± 30 sec, τ_V = 690 ± 60 sec, τ_{F_1} = 230 ± 20 sec, τ_{F_2} = 960 ± 120 sec. Values of $\dot{V}_{max}$ established under the influence of different concentrations of ethmozine DAA are shown in Fig. 3a.

Changes in myocardial electrical activity were accompanied by a fall in the force of contraction (Fig. 1a, b). The process of inhibition of the force of contraction by ethmozine

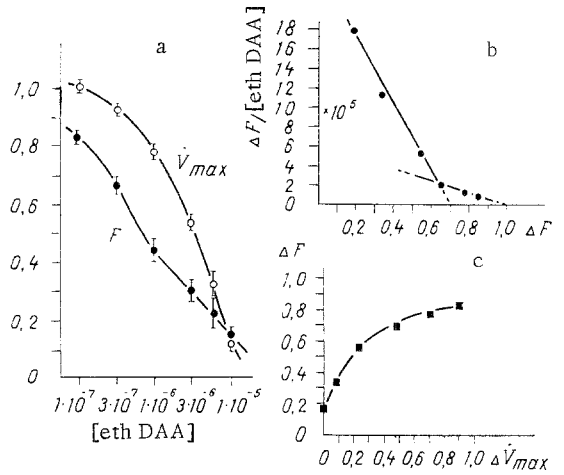

Fig. 3. Analysis of steady-state values of $\dot{V}_{max}$ and force of contraction in various concentrations of ethmozine DAA. a) Growth-effect curves (empty circles show $\dot{V}_{max}$, filled circles show force of contraction) for relative values of recorded parameters; b) representation of dependence of change in force of contraction on concentration of ethmozine DAA in Scatchard coordinates. Approximating lines in the general form have the equation $\Delta F = \Delta F_{max}/\{1 + K_{DF}/[\text{ethmozine DAA}])\}$, where $\Delta_{F_{max}}$ is the maximal inhibition of the contraction, and K_{DF} is the apparent dissociation constant of ethmozine DAA (values of $\Delta_{F_{max}}$ and K_{DF} are given in Table 2). The points are taken from the graph in Fig. 3a; c) dependence of relative decrease in force of contraction ($\Delta_F = 1 - F$) on relative decrease in $\dot{V}_{max}$ ($\Delta\dot{F}_{max} = 1 - \dot{V}_{max}$), where F and $\dot{V}_{max}$ are taken from the graph in Fig. 3a.

DAA (Fig. 2b) is approximated best of all by two exponents with different time constants. During the first 2-3 min the fall in the force of contraction was not yet accompanied by any appreciable decrease in $\dot{V}_{max}$ (Fig. 1a; Fig. 2a) and it took place relatively quickly. A further decrease in the force of contraction developed much more slowly (Fig. 2b); the time constant of the fall in $\dot{V}_{max}$ under the influence of the same concentration of ethmozine DAA was similar (but a little smaller) to the time constant of the second phase of fall of the force of contraction under the influence of ethmozine DAA. The decrease in the force of contraction in the presence of other concentrations of ethmozine DAA developed similarly.

These data suggest that the second phase of a decrease in the force of contraction is most probably connected with blocking of the fast sodium channels, reflected in a decrease in $\dot{V}_{max}$ and, consequently, a decrease in the supply of Na^+ ions to the cardiomyocytes and inhibition of sodium-calcium exchange. This hypothesis is based on the fact that blocking of the fast sodium current alone by tetrodotoxin [2, 14] or by ethmozine [1] correlates well with the decrease in the force of contraction, and this can only be explained by inhibition of the sodium-calcium exchange mechanism and a decrease in the number of Ca^{++} ions entering the cell by this mechanism with each excitation cycle. However, the decrease in the force of contraction under the influence of ethmozine and tetrodotoxin was monoexponential, for these compounds affect only the fast sodium current [1, 2].

To determine the cause of the appearance of the rapid phase of fall of the force of contraction under the influence of ethmozine DAA, the effect was studied on the Ca-AP, the overshoot of which is mainly determined by the small calcium current [2]. It was found that with high concentrations of ethmozine DAA the Ca-AP overshoot decreases (Table 1). The development of this process in time with ethacizine (ethmozine DAA) in a concentration of 3×10^{-6} g/ml is illustrated in Fig. 2a, 2. The mean value of the time constant of fall of the Ca-AP overshoot (220 ± 30 sec) is close to the time constant of the first phase of all of the force of contraction under the influence of ethmozine DAA. It can accordingly be concluded that the rapid initial inhibition of the force of contraction under the influence of ethmozine DAA, unaccompanied by any appreciable change in $\dot{V}_{max}$ of the normal AP, is determined by blocking of the slow calcium current, which is manifested as a decrease in the Ca-AP overshoot.

TABLE 1. Changes in Ca-AP Overshoot (in mV) under the Influence of Different Concentrations of Ethmozine DAA

Change in overshoot	Concentration of ethmozine DAA, g/ml					
	$3 \cdot 10^{-8}$	$1 \cdot 10^{-7}$	$3 \cdot 10^{-7}$	$1 \cdot 10^{-6}$	$3 \cdot 10^{-6}$	$1 \cdot 10^{-5}$
M	+0,1	+1,5	—0,5	—2,5	—4,0	—6,0
$\pm m$	0,3	0,5	0,6	0,7	1,1	0,9
n	3	4	4	3	5	4

TABLE 2. Apparent Dissociation Constants of Ethmozine dAA on Myocardium Obtained by Scatchard Plot Analysis of Dose—Effect Curves for V_{max} and Source of Contraction

Value recorded	Force of contraction (1)	Force of contraction (2)	$\dot{V}_{max}$
Dissociation constant (K_D), g/ml	$3{,}03 \cdot 10^{-7}$	$1{,}54 \cdot 10^{-6}$	$3{,}05 \cdot 10^{-6}$
Maximal inhibition	0,7	1,0	1,0

It thus seems that there are two sites of action of ethmozine DAA on the working mammalian myocardium: action on sodium and on calcium channels. This conclusion is confirmed by investigation of dose—effect curves (Fig. 3a). Analysis of the curve of force of contraction as a function of ethmozine DAA concentration in Scatchard coordinates [13], which as the graph in Fig. 3d shows is best approximated by two straight lines, demonstrates that interaction between ethmozine DAA and myocardium is described by two apparent binding constants, i.e., that there are two sites of action of ethmozine DAA on the myocardium. Dependence of V_{max} on ethmozine DAA concentration in a Scatchard plot is represented by only one apparent binding constant (Table 2).

Dependence of the relative value of the decrease in force of contraction under the influence of ethmozine DAA on the relative value of the decrease in $\dot{V}_{max}$, moreover, is nonlinear (Fig. 3c). This result differs in principle from the analogous relationships obtained during the action of tetrodotoxin and ethmozine [1, 2], and this can be explained by the existence of two sites of action of ethmozine DAA on the myocardium. It was shown that the calcium current is more sensitive to the action of ethmozine DAA than the sodium current (Table 2, values of K_D), and for that reason the relationship illustrated in Fig. 3c arises more sharply to begin with, i.e., in the presence of low concentrations of ethmozine DAA, when $\dot{V}_{max}$ has still changed only a little, and the fall in the force of contraction is mainly due to blocking of the calcium current.

The results of this investigation thus confirm the previous hypothesis that a reduction in the entry of Na^+ ions into cardiomyocytes along fast sodium channels can give rise to a negative inotropic effect, on account of inhibition of sodium-calcium exchange [9, 11]. In addition, the negative inotropic effect of ethmozine DAA is partly due to blocking of the slow calcium current.

The data described above, which were obtained on isolated papillary muscle preparations, evidently reflect only the potential capacity for a change in the inotropic state of the myocardium under the influence of ethmozine DAA. Under experimental conditions *in vivo* [5] and also under clinical conditions [7, 8] ethmozine DAA caused no significant changes in cardiac contractility. The reasons for the difference in the responses of the myocardium to ethmozine DAA in experiments *in vivo* and *in vitro* are not known and require further study.

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EFFECT OF SEROTONINERGIC AGENTS ON AVOIDANCE BEHAVIOR UNDER ACUTE STRESS

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Activation of the serotoninergic system of the brain is accompanied by depression of behavioral responses [4, 6]. Conversely, a fall in the brain serotonin (5-HT) level correlates with increased motor reactivity and the performance of purposeless, superfluous responses [11]. However, in the initial stages of habit formation elevation of the brain 5-HT level reduces emotional hyper-reactivity and improves learning [2]. The role of the serotoninergic system in adaptation to emotional stress has not been adequately studied.

The object of this investigation was to assess the effect of drugs with serotonin positive and antiserotonin activity on realization of avoidance behavior in a stress situation under conditions of a relative deficiency of information on the method of avoidance.

EXPERIMENTAL METHOD

Male Wistar rats weighing 200-250 g, kept in cages, six animals in each cage, with free access to food and water, were used. Avoidance behavior was assessed by the method [10] in the modification [1]. All drugs were injected intraperitoneally in aqueous solutions. The following drugs were used: 5-hydroxytryptophan (5-HTP, from Serva, West Germany), zimelidine (provided by Professor Ross, from Astra), trazodone and m-chlorophenylpiperazine — CPP (provided by Professor Maj, Institute of Pharmacology, Polish Academy of Sciences, Cracow), pirenpiron (supplied by Professor R. Janssen, Janssen Pharmaceutica), cyproheptadine (from Serva), quipazine (S. Ordzhonikidze All-Union Pharmaceutical Chemical Research Institute, supplied by Professor N. N. Suvorov). The results were subjected to statistical analysis by Student's t test.

EXPERIMENTAL RESULTS

Data on the time course of avoidance behavior under acute emotional stress, before and after administration of the serotoninergic drugs, are given in Table 1. In agreement with previous observations [1], the first time the untrained animal was placed in a glass cylinder fixed in a vessel of water, so that the only way of avoidance was by diving, the rats developed an emotional stress response, largely because of lack of information on the method of avoidance.

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