ANALYSIS OF CHANGES IN DURATION OF ACTION POTENTIAL OF MYOCARDIAL CELLS DURING LOCAL ANAPHYLACTIC REACTION AND IN RESPONSE TO HISTAMINE IN GUINEA PIGS

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The dependence of the duration of the action potential (AP) on the frequency of spontaneous activity during the local anaphylactic reaction (antigen egg albumin, $2 \cdot 10^{-4}$-$2 \cdot 10^{-5}$ g/ml) and in response to histamine ($1 \cdot 10^{-4}$-$1 \cdot 10^{-5}$ g/ml) was investigated on the right atrial auricle of guinea pigs under the following experimental conditions: in normal Tyrode solution, b) during blocking of the CA channels by verapamil (2-4 mg/liter) or D-600 (0.2-1 mg/liter) and c) during blocking of the potassium channels by tetraethylammonium (10-20 mM). Analysis of interspike interval versus AP during clots revealed a direct prolonged effect of the antigen and histamine on the duration of the AP plateau, which was masked by the positive chronotropic effect of these agents. Relative lengthening of AP took place over the whole range of frequencies. The results confirm the previous hypothesis of activation of the slow Na–Ca channels during the local anaphylactic reaction and in response to the action of histamine on the myocardium.

KEY WORDS: anaphylaxis; histamine; myocardium; action potential.

Previous experiments on preparations of the auricle of the guinea pig atrium depolarized by means of 20 mM KCl showed [1, 5] that during the local anaphylactic reaction to egg albumin and also during the action of histamine the duration of the plateau of the evoked action potential (AP) is considerably increased. Since blocking of the slow Na–Ca channels of myocardial cells by means of verapamil or compound D-600 weakened these effects of histamine and of the antigen, it has been suggested that activation of the Na–Ca channels takes place during the action of these agents. There are individual statements in the literature to the effect that antigen and histamine prolong the plateau of AP of spontaneous contracting preparations of the mammalian heart in normal salt medium [3, 2-4, 9, 10]. However it is not known at what frequency of spontaneous activity APs with increased duration were recorded. However, it is known that histamine and antigen (in sensitized animals) have a marked chronotropic action [3-5, 10]; an increase in frequency, however, must lead to regular shortening of AP duration [6, 11, 12]. In order to distinguish the direct effect of these agents on the duration of the AP plateau from their effect on this plateau through a change in frequency, in the present investigation the dependence of AP duration on the preceding interspike interval was studied over a wide range of spontaneous frequencies: under normal conditions, during the local anaphylactic reaction, after addition of histamine to the Tyrode solution, and after addition of blocking agents.

EXPERIMENTAL METHOD

Experiments were carried out on the isolated, spontaneously contracting auricle of the guinea pig atrium. The animals were sensitized to egg albumin by the scheme of Feigin et al. [10]. The AP was recorded by intracellular glass microelectrodes filled with 3 M KCl, and contraction was recorded isometrically by means of the 6MKhIS mechanotron. Throughout the experiment the auricle was perfused with Tyrode solution, to which the blocking agents and test substances were successively added. The solution was saturated with a gas mixture consisting of 96% O_2 and 4% CO_2. The temperature of the solution was maintained between 34 and 36°C and the pH between 7.2 and 7.4. The duration of the AP was measured at the level of one-third (A) and two-thirds (B) of its amplitude. For each series of experiments graphs of interspike interval versus AP duration (at two levels) were plotted: for background effects (Tyrode solution, substances blocking slow Na–Ca channels, tetraethylammonium – TEA) and for histamine and the antigen. Such graphs can be used to study the action of the test agents and its dependence on blocking agent for any interspike interval chosen.

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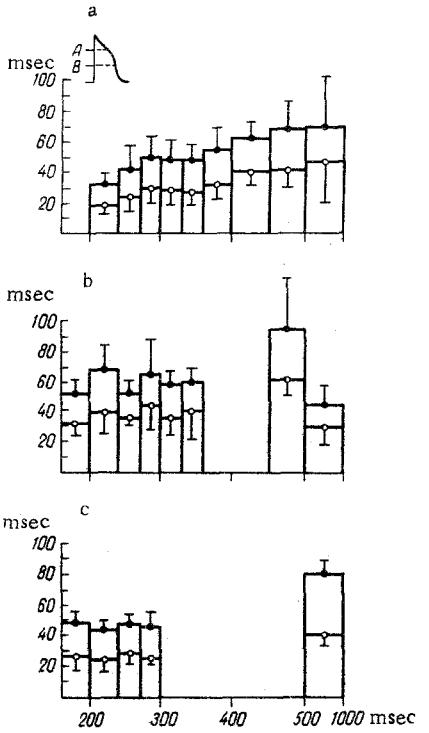

Fig. 1. Duration of AP as a function of interspike interval in tyrode solution (a), after addition of antigen in concentration of $2 \cdot 10^{-4}$-$2 \cdot 10^{-5}$ g/ml (b), and histamine in concentrations of $1 \cdot 10^{-4}$-$1 \cdot 10^{-5}$ g/ml (c). Series (b) carried out on preparations of atrial auricle of previously sensitized guinea pigs. On all graphs top curves reflect changes in AP duration at B level, bottom curves – at A level. Abscissa, duration of interspike intervals (in msec); ordinate, duration of AP (in msec).

EXPERIMENTAL RESULTS AND DISCUSSION

The relationship between the AP duration and length of the interspike interval in Tyrode solution is shown for 359 cells in 68 experiments in Fig. 1a. The smooth rise of AP duration with an increase in interspike interval will be noted. In 51% of cells studied the duration of the interspike intervals was 360-400 msec. After addition of the antigen (87 cells) and of histamine (33 cells) an increase in the frequency of spontaneous activity was observed, and this was reflected on the graph as a shift of the range of interspike intervals to the left (Fig. 1b, c). In most cells the interspike interval did not reach 360 msec (86 and 90% of all cells treated with antigen and with histamine respectively). Despite this marked chronotropic effect, broadening of the AP plateau was clearly defined, as will be clear from Fig. 1. In response to both factors the broadening was statistically significant ($P < 0.01$ or $P < 0.05$) within the range of interspike intervals from 200 to 270 msec. As the intensity of the effect fell at interspike intervals of over 300 msec, the broadening of the plateau ceased to be significant.

The action of verapamil (2-4 mg/liter) and compounds D-600 (0.2-1 mg/liter), which blocks slow Na–Ca channels, on the spontaneously contracting preparations led to a gradual decrease in the frequency of spontaneous activity. Of the 177 cells recorded in 20 experiments, in 77 (57%) the interspike intervals were distributed within the range from 450 to 2000 msec (Fig. 2a). In normal Tyrode solution (Fig. 1a) the duration of AP rose steadily with an increase in the interspike interval. Blocking of the Na–Ca channels caused a decrease in the duration of AP ($P < 0.01$) at high interspike intervals (from 500 to 2000 msec) compared with the characteristic level for the corresponding frequency ranges under normal conditions (compare the graphs 1a and 2a). The

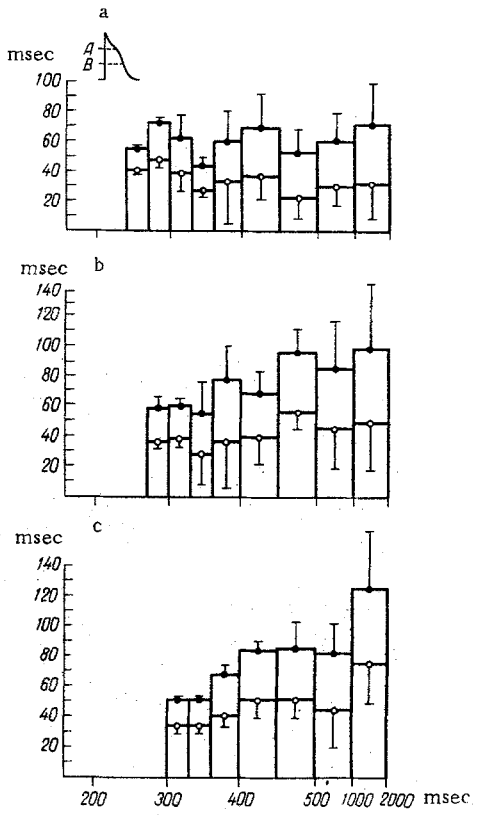

Fig. 2. Duration of AP as a function of duration of interspike interval during inhibition of slow Na–Ca channels by verapamil in concentration of 2-4 mg/liter or by compound D-600 in concentration of 0.2-1 mg/liter (a) and after addition of antigen to these solution in concentration of $2 \cdot 10^{-4}$-$2 \cdot 10^{-5}$ g/ml (b) and addition of histamine in concentrations of $1 \cdot 10^{-4}$-$1 \cdot 10^{-5}$ g/ml (c). Series (b) carried out on preparations of atrial auricle of previously sensitized guinea pigs. Remainder of legend as in Fig. 1.

agent blocking the slow Na–Ca channels thus gave two effects; bradycardia and narrowing of the AP; both these effects developed parallel to one another in time.

After superposition of the action of the antigen and histamine on the action of the agents blocking the slow Na–Ca channels no redistribution of interspike intervals took place: most cells (73 and 57% respectively) had interspike intervals within the range from 450 to 2000 msec. The antigen, like histamine, when superposed on the action of agents blocking Na–Ca channels, induced widening of AP (Fig. 2b, c), which led to restoration of the linear relationship between the duration of AP and the duration of the interspike interval as observed previously in Tyrode solution and disturbed by the blocking agents. The data on the broadening of AP under the influence of antigen and histamine, superposed on the action of the agents blocking Na–Ca channels are statistically significant for interspike intervals between 450 and 2000 msec ($P < 0.01$).

In a special series of experiments the effect of antigen and histamine on the duration of AP was investigated during blocking of the K channels by means of TEA. In concentrations of 10-20 mM, TEA depresses the outward current of K ions by 60-90%, as has been shown on frog skeletal muscles [15]. In the five experiments now carried out to study the action of TEA in these concentrations bradycardia developed, and this was accom-

TABLE 1

External conditions	Duration of AP, msec	
	level A	level B
Tyrode solution	30,2±10,7	49,2±14.3
TEA	43,5±1,5	65,5±2,6
TEA + histamine	58,9±2,2	87.4±1.8

panied by parallel widening of AP. However, when comparing the duration of AP associated with identical interspike intervals, the writer concluded that the increase in the duration of AP under the influence of TEA is not the result of bradycardia. As an example, values of the duration of AP for normal conditions, TEA treatment, and the combined action of TEA and histamine over the range of interspike intervals from 300 to 350 msec are given in Table 1.

In normal Tyrode solution a frequency of this range was recorded in only 22% of cells (79 of 359); as a result of the development of bradycardia during the action of TEA the number of cells with this frequency fell even more – to 12% (4 of 37 cells); however, after the addition of histamine to the TEA solution the frequency increased again, and 38% of cells were within this range (14 of 37). It will be clear from Table 1 that the duration of AP during the action of TEA was longer than normal ($P < 0.1$); the addition of histamine to the TEA solution caused an even further increase in the duration of AP ($P < 0.01$).

Analysis of the dependence of the duration of AP on the interspike interval over the whole spectrum of frequencies thus gave statistically significant confirmation of the previous data on the broadening of AP during local cardiac anaphylaxis and during the action of histamine [1, 3-5, 8, 10]. It was shown that the effect of AP broadening under these conditions is independent of the presence or absence of the chronotropic action of the agents. Manifestation of the AP broadening effect is prevented neither by blocking of the slow Na–Ca channels nor blocking of the outward potassium current. The blocking of these systems by itself varies the duration of AP of the myocardial cells in different ways: blocking Na–Ca channels shortens AP, whereas blocking of the K channels lengthens it. Antagonism between the actions of factors blocking slow Na–Ca channels and the effect of antigen and histamine on the duration of AP confirms the hypothesis put forward originally by the present writer [1, 5, 7, 8] and subsequently by other workers [13, 14], that the phenomenon described is based on activation of slow Na–Ca channels.

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