PRESYNAPTIC ACTION OF IMIDAZOLE ON MYONEURAL JUNCTIONS IN FROGS

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Imidazole (9 mmoles/liter) increased the degree of neuromuscular depression determined from the reduction in amplitude of the second end-plate potential during stimulation with paired pulses separated by an interval of 1 sec. During repetitive stimulation imidazole reduced the degree of potentiation of the end-plate potential, thus facilitating the development of subsequent depression, especially during stimulation at a frequency of 100/sec. The absolute refractory period of the end-plates was reduced.

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Imidazole is represented in the skeletal muscles of vertebrates as the natural compounds carnosine and anserine [7, 8, 10]. Numerous investigations [1-3, 8-10] have shown that imidazole can restore the working capacity of a fatigued frog nerve-muscle preparation during indirect stimulation, and can also abolish the block to neuromuscular transmission in cold-blooded and warm-blooded animals during curarization. The effect of imidazole on a nerve-muscle preparation during indirect stimulation under conditions of fatigue or curarization has been explained by its ability to act on the postsynaptic membrane and to increase the sensitivity of cholinergic receptors to acetylcholine [2, 10, 11]. Experiments using microelectrodes have shown that imidazole can also exert a presynaptic action, increasing the quantum composition of the end-plate potentials (EPP). In the modern view [16], the mean number of quanta of mediator (m) liberated by a nervous impulse depends on the total number of quanta of accessible mediator in the presynaptic ending (n) and the probability of liberation of each quantum (P):

$$m = nP.$$

Consequently, an increase in the quantum composition of the EPP (m) by the action of imidazole may take place through an increase in either n or P. The present investigation was carried out to investigate this problem.

EXPERIMENTAL METHOD

Experiments were carried out on nerve-muscle preparations of the sartorius muscle of pond frogs. During the experiment the preparation was kept in a bath with a continuous flow of Ringer's solution, which could quickly be replaced by Ringer's solution containing imidazole in a concentration of 9 mmoles/liter. The EPP was recorded by means of glass microelectrodes filled with 2.5 M KCl solution and with an impedance of between 6 and 20 MΩ. The sciatic nerves were stimulated by means of an electronic stimulator with radiofrequency output. The EPP was recorded after curarization (D-tubocurarine chloride, $1 \cdot 10^{-6}$ - $4 \cdot 10^{-6}$ g/ml) on photographic film from the screen of a CRO after amplification by means of a dc amplifier. Experiments were carried out at room temperature (20-22°), and the pH of the solutions was kept at 7.3-7.4. To determine whether imidazole increases the total number of quanta of mediator in the presynaptic endings (n) or increases the probability of liberation of the quantum (P), the following method was used [17, 18]. During indirect stimulation of a nerve-muscle preparation after curarization by two stimuli separated by an interval of 1 sec, the amplitude of the second EPP is always less than the amplitude of the first [12, 15, 19]. This has been explained by partial exhaustion of reserves of accessible mediator by the first impulse [14]. If imidazole increases the quantum composition of the EPP (m) by increasing the prob-

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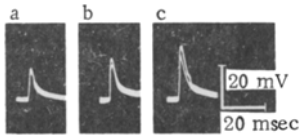

Fig. 1. Effect of imidazole on degree of neuromuscular depression. EPP before action (a) and after 3 (b) and 5 (c) min of action of imidazole. Paired stimulation with interval of 1 sec. First and second EPPs are superposed on each frame. Preparation kept in Ringer's solution with increased concentration of calcium ions.

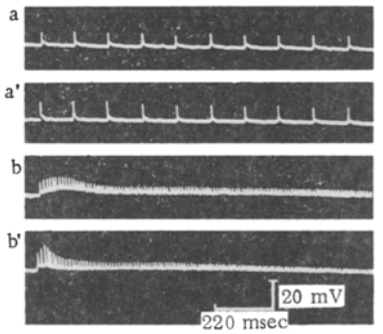

Fig. 2. Effect of imidazole on development of EPP during repetitive stimulation. EPP before action (a, b) and after 5 min (a', b') of action of imidazole during stimulation at frequencies of 10 and 100 pulses/sec. Preparation kept in Ringer's solution of normal ionic composition.

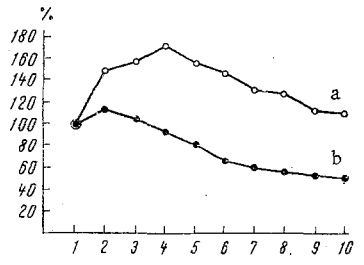

Fig. 3. Facilitation and depression of neuromuscular transmission during repetitive stimulation (frequency of stimulation 100/sec). a) Before action of imidazole; b) after 5 min of action of imidazole. Abscissa, order of EPP in repetitive series; ordinate, amplitude of successive EPPs relative to amplitude of the first EPP (in %).

ability of liberation of the quantum of mediator (P), the decrease in amplitude of the second EPP (relative to the amplitude of the first EPP) will become still more marked, because the first impulse will produce greater exhaustion of the reserves of accessible mediator. If, on the other hand, imidazole influences the number of quanta of accessible mediator (n) in the presynaptic ending, the relationship between the amplitudes of the first and second EPP will not be changed. Later in the text the decrease in amplitude of the second EPP relative to the amplitude of the first EPP will be described as neuromuscular depression [17, 18]. The degree of neuromuscular depression was calculated from the formula [17, 18]:

$$\frac{V_1 - V_2}{V_1} \cdot 100\%,$$

where V_1 is the amplitude of the first EPP and V_2 the amplitude of the second EPP. To increase the degree of neuromuscular depression in a considerable proportion of the experiments Ringer's solution with an increased concentration (up to 12.6 mmoles/liter) of calcium ions was used [17]. Paired pulses separated by an interval of 1 sec were applied to the nerve every 30 sec. Repetitive stimulation at different frequencies was carried out at intervals of 1-2 min.

EXPERIMENTAL RESULTS

During paired stimulation of the nerve-muscle preparation, imidazole appreciably increased the amplitude of the first EPP 1-3 min after its administration, and at the same time it produced deeper depression of the second EPP. After the action of imidazole for 5 min the amplitude of the first EPP and, correspondingly, the degree of neuromuscular depression as a rule reached a maximum (Fig. 1a, b, c). If the degree of neuromuscular depression before administration of imidazole was 16.4±1.5%, after its action for 3 and 5 min the degree of neuromuscular depression was increased to 21.4±1.2 and 24.2±1.8%, respectively ($P < 0.05$).

Consequently, imidazole did not affect the total reserve of accessible mediator in the presynaptic nerve endings (n), but increased the probability of liberation of mediator by the nervous impulse (P).

During repetitive stimulation (10 and 100 pulses/sec) of the nerve-muscle preparation in Ringer's solution with normal ionic composition imidiazole caused an appreciable increase in amplitude of the first EPP in each series of stimulations. However, after brief and slight potentiation, deeper than normal depression of the EPP was observed (Figs. 2a, a', b, b'). Measurement of 10 successive EPPs (expressed in percentages of the first EPP) during stimulation at 100/sec showed that the amplitude of the EPP reached a maximum in response to the fourth pulse, when its value was 171.4% (Fig. 3a). This agrees with earlier investigations [6]. After the action of imidazole for 5 min, the maximum was reached not with the fourth, but with the second EPP, but its amplitude was only 116.5% relative to the first EPP (Fig. 3b). It was followed by a sharp decrease in amplitude of the successive EPPs, and the 10th EPP was only 46.5% of the amplitude of the first EPP (compared with 105% normally). The results of experiments with repetitive stimulation also indicate that imidazole increased the probability of liberation of mediator. In fact, if the first pulse largely exhausted the reserves of accessible

mediator, the conditions providing for maintenance of the normal degree of potentiation of successive EPPs through mobilization of mediator were no longer present.

Furthermore, every successive pulse produced still greater exhaustion of the reserves of accessible mediator, thus reducing the liberation of quanta of mediator and producing marked depression of the EPP.

In these experiments imidazole as a rule increased the amplitude of the membrane potential by several millivolts, but these differences were not significant.

The effect of imidazole on the duration of the absolute refractory phase (ARP) of the end plate also was investigated. The ARP of the end plate was revealed by omission of the second EPP during indirect stimulation of the nerve-muscle preparation by paired stimuli separated by a short interval, and was none other than the ARP of the presynaptic endings of the axon [4, 5, 13]. After 5 min of its action, imidazole slightly reduced the ARP of the end-plate, on the average from 2.07 msec to 1.72 msec ($P < 0.05$).

These experiments thus showed that imidazole has a marked presynaptic action. It increased the quantum composition of the EPP by increasing the probability of liberation of mediator by the nervous impulse from the presynaptic nerve endings. The marked decrease in ARP of the end-plate during the action of imidazole indicated that its effect was not limited to its action on the mechanism of liberation of mediator from presynaptic nerve endings.

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