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Barbiturates increase the rate of voltage-dependent inactivation of the calcium current in snail neurones

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1 Effects of barbiturates (thiopentone, pentobarbitone, phenobarbitone and barbitone) on the calcium current (I_{Ca}) in identified *Helix* neurones were studied, using a conventional suction pipette technique.

2 Barbiturates depressed the maximal peak amplitudes (MPA) of I_{Ca} in a dose-dependent manner without shifting the current-voltage relationships along the voltage axis.

3 Barbiturates accelerated the decay phase of I_{Ca} at high concentrations (1×10^{-4} to 3×10^{-3} M), at which concentrations double-pulse experiments showed the increased rate of a voltage-dependent inactivation of I_{Ca}.

4 It is concluded that the acceleration of the decay phase of I_{Ca} by barbiturates may be due to the increased rate of the voltage-dependent inactivation of I_{Ca}.

Introduction

We have previously shown that pentobarbitone accelerates the decay phase of the calcium current (I_{Ca}) in *Helix* neurones (Nishi & Oyama, 1983). The accelerating action of pentobarbitone on the decay phase of I_{Ca} was reminiscent of the accelerated decay phase of the sodium current (I_{Na}) seen with the internal presence of pancuronium molecules (Yeh & Narahashi, 1977). The acceleration of the decay phase of I_{Na} by pancuronium appears to be due to a time-dependent blockade of open sodium channels by drug molecules rather than to an increased rate of the voltage-dependent inactivation of I_{Na}. Their conclusion was based on experiments in which the rate of the voltage-dependent inactivation was determined by the double-pulse method with small depolarizing prepulses which did not induce inward current (Yeh & Narahashi, 1977). The inactivation of I_{Ca} in molluscan neurones is not only voltage-dependent but also current-dependent (Brehm & Eckert, 1978; Tillotson, 1979; Brehm, Eckert & Tillotson, 1980; Brown, Morimoto, Tsuda & Wilson, 1981; Plant & Standen, 1981). Therefore, explanations for the mode of the accelerating action of pentobarbitone on the decay phase of I_{Ca} must be more complicated than those for pancuronium on the I_{Na} (Nishi & Oyama, 1983).

The first aim of the present experiments was to distinguish, by using the double-pulse method, whether the effects of pentobarbitone on the time course of I_{Ca} depend on the voltage-dependent inactivation or on the current-dependent inactivation and/or open calcium channel. The second aim was to characterize the mode of action of pentobarbitone on the decay of I_{Ca}; we studied the structure-activity relationship in the accelerating action of barbiturates on the decay phase of I_{Ca}, with regard to the depressant action on the maximal peak amplitude of I_{Ca}.

Preliminary results have been given elsewhere (Oyama, Nishi & Akaike, 1983a).

Methods

The experimental method was essentially similar to that previously described by Lee, Akaike & Brown (1980). Experiments were performed on intracellularly-perfused, identified giant neurones (F–1 cell; Kerkut, Lamber, Gayton, Locker & Walker, 1975), using a conventional suction pipette method which allows voltage clamp and internal perfusion. In brief, the suboesophageal ganglion of the snail, *Helix aspersa*, was removed and the connective tissue was stripped off with fine forceps. A part of an individual neurone (about 150 μm diameter) was aspirated under negative pressure of about −300 mmHg so as to occlude the tip (about 20 μm diameter) of a suction pipette.

The calcium current (I_{Ca}) was separated by blockade of the potassium and sodium currents by sub-

stitution of $Tris^+$ and tetraethylammonium (TEA^+) for Na^+ and of Cs^+ for K^+ in the internal and external solutions. The ionic composition of the test solutions was as follows: the internal solution (mM), Cs aspartate 135, TEA OH 10 and EGTA acid 1 at pH 7.4 buffered by HEPES 5 mM and excess aspartic acid; the external solution (mM), Tris Cl 30, TEA Cl 50, CsCl 5, $CaCl_2$ 10, $MgCl_2$ 15, 4-aminopyridine 5 and glucose 5.5 I_{Ca} was monitored on a storage oscilloscope (Tektronix, 5113), and simultaneously recorded with a photosensitive paper recorder system (Medelec, MS6). The transient linear leakage and capacitive currents were subtracted by adding the currents produced by each of a pair of depolarizing and hyperpolarizing pulses of equal amplitude by a signal averager (Nihonkoden, ATAC-150).

Barbiturates were dissolved in the external solution at appropriate concentrations just before use. Barbiturates used were thiopentone (Midorijuji, Japan), pentobarbitone (Tanabe Pharmac., Japan), phenobarbitone (Yamazen Pharmac., Japan) and barbitone (Wako Chemical, Japan). All experiments were carried out at room temperatures (24–25°C).

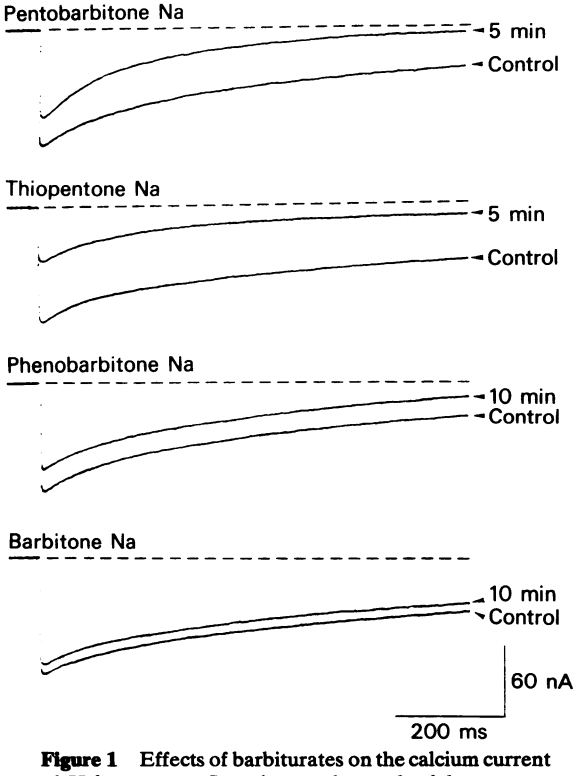

Figure 1 Effects of barbiturates on the calcium current of *Helix* neurone. Superimposed records of the currents before (control) and 5 to 10 min after the start of external applications of barbiturates (5 to 10 min) at a concentration of 1×10^{-3} M. The membrane was clamped at the holding potential of −50 mV to a voltage step of +20 mV.

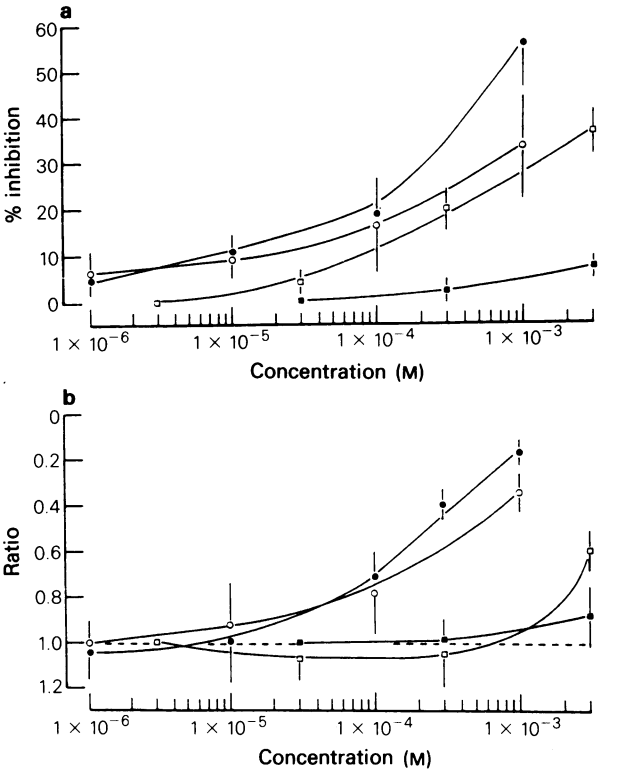

Figure 2 (a) Dose-response curves for the depressant action of barbiturates on the maximal peak amplitude of the calcium current 10 min after applications of barbiturates at various concentrations: (○) pentobarbitone; (●) thiopentone; (□) phenobarbitone; (■) barbitone. (b) Dose-response curves for the accelerating action of barbiturates on the decay phase of the current. Symbols as in (a). Vertical bars at each point in (a) and (b) indicate s.d. of four experiments.

Results

Effects of barbiturates on the calcium current

The membrane potential of the neurone was clamped at a constant holding potential of −50 mV and depolarizing steps of +60 to +70 mV to obtain the maximal peak amplitude (MPA) were applied to the preparation in the presence and absence of barbiturates. The drugs gave maximal inhibition on I_{Ca} within 5 to 10 min after beginning the application. In the following experiments, therefore, the effects of barbiturates on I_{Ca} were measured 10 min after the start of drug application. Thiopentone, pentobarbitone

and phenobarbitone at a concentration of 1×10^{-3} M depressed the MPA of I_{Ca} to 50–80% of the control, but barbitone (1×10^{-3} M) slightly reduced the MPA of I_{Ca} (Figure 1). These depressant actions of barbiturates on the MPA of I_{Ca} occurred without shifting the current-voltage relationships along the voltage axis. Dose-response curves for the depressant action of barbiturates on the MPA of I_{Ca} were obtained by calculating the percentage inhibition of the MPA by the agents at various concentrations relative to the control (Figure 2a). The potency of the depressant action of barbiturates on the MPA was in the order: thiopentone, pentobarbitone, phenobarbitone, barbitone.

Pentobarbitone markedly accelerated the decay phase of I_{Ca} (Nishi & Oyama, 1983). Thiopentone also markedly accelerated the decay phase at a concentration of 1×10^{-3} M, but the effects of phenobarbitone and barbitone on the decay phase of I_{Ca} were not very pronounced at this concentration as compared with those of pentobarbitone and thiopentone (Figure 1). Dose-response curves for the accelerating action of barbiturates on the decay phase of I_{Ca} were obtained by calculating the ratio of the half decay time (the time required for decreasing the current amplitude to half of the MPA from the peak of the current) in the presence of the agents at various concentrations relative to those of the control (Figure 2b). Pentobarbitone and thiopentone decreased the ratio of the half decay time in a concentration-dependent manner, while phenobarbitone and barbitone only at high concentrations exhibited the obvious accelerating action on the decay phase of I_{Ca} (Figure 2b).

Double-pulse experiments on the voltage-dependent inactivation of the calcium current

The steady-state level of the voltage-dependent inactivation of I_{Na}, determined by the double-pulse

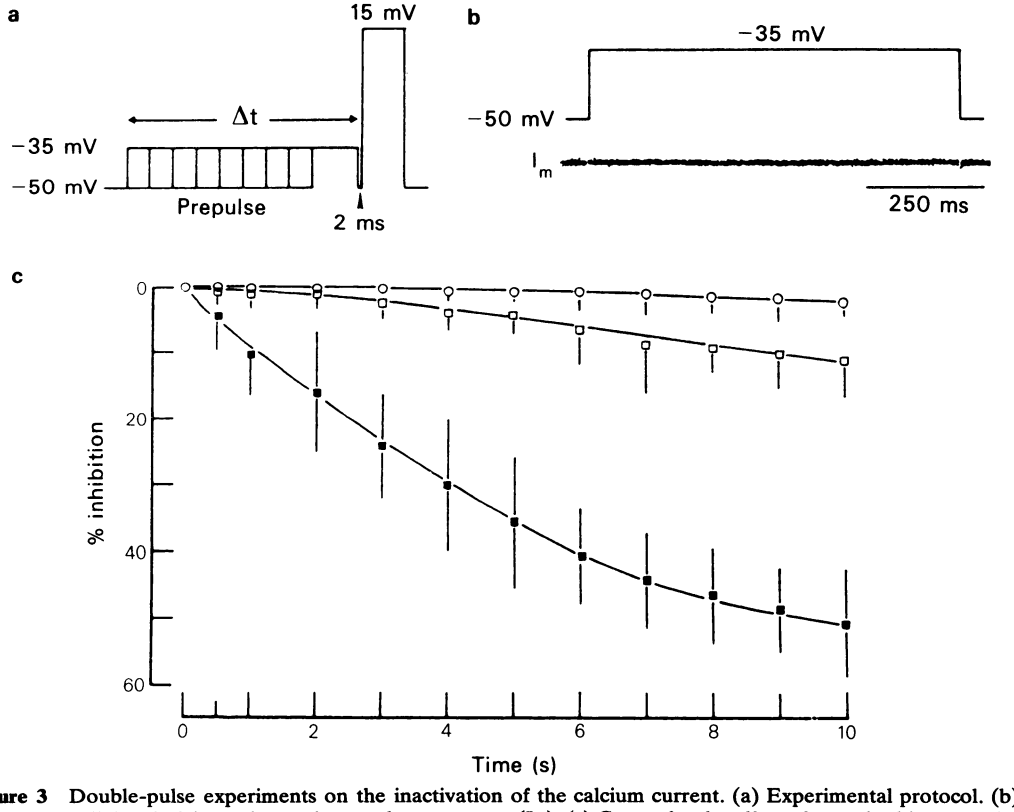

Figure 3 Double-pulse experiments on the inactivation of the calcium current. (a) Experimental protocol. (b) Effect of small depolarizing pulse on the membrane current (I_m). (c) Curves for the effect of pentobarbitone on the steady-state level of inactivation of the calcium channel: (○) control; pentobarbitone 1×10^{-4} M (□), 1×10^{-3} M (■). The level of inactivation reached during the prepulses of various durations was represented by the percentage inhibition of peak amplitude of the current induced by the test pulse. Vertical bars at each point indicate s.d. in three experiments.

method for small depolarizations which hardly open the sodium channel, was not affected by pancuronium, a blocker of open sodium channels (Yeh & Narahashi, 1977). To examine whether barbiturates would act as a blocker of open calcium channels, we designed the experimental protocol for I_{Ca} shown in Figure 3a. Since the membrane was depolarized from its holding potential of -50 mV to -35 mV at which level any inward currents were not induced (Figure 3b), we assumed that there would be very little opening of calcium channels in this voltage condition. The level of voltage-dependent inactivation reached during the prepulse was assayed by the peak amplitude of I_{Ca} induced by the test pulse to $+15$ mV. The curves for the effects of barbiturates on the voltage-dependent inactivation level of I_{Ca} were obtained by calculating the percentage inhibition of the peak amplitude of I_{Ca} induced by the test pulse with the prepulse, relative to that induced by the test pulse without the prepulse (Figure 3c). In the control period, the level of voltage-dependent inactivation was little affected by long depolarizing prepulses, while in the presence of pentobarbitone the peak amplitude of I_{Ca} induced by the test pulse with the prepulse decreased, depending upon the duration of the prepulse (Figure 3c). With the prepulse lasting for 10 s in the presence of pentobarbitone 1×10^{-3} M, the peak amplitude of I_{Ca} induced by the test pulse was reduced to about 50% of the control. Other barbiturates also showed the decrease in the peak amplitude induced by the test pulse at higher concentrations than 1×10^{-3} M (not illustrated).

Discussion

In the present experiments, the barbiturates used reduced the MPA of I_{Ca} in a concentration-dependent manner and accelerated the decay phase at high concentrations. The potency order of the depressant action on the MPA and the accelerating action of barbiturates was similar. From the results of double-pulse experiments on the inactivation level of I_{Ca}, the acceleration of the decay phase of I_{Ca} in the presence of barbiturates may be concluded to result from the increased rate of the voltage-dependent inactivation of I_{Ca} in the perfused snail neurone rather than a time-dependent blockade of the open calcium channel. Both thiopentone and pentobarbitone had an obvious accelerating action on the decay phase of I_{Ca} at a concentration of 1×10^{-3}M, while phenobarbitone and barbitone had little accelerating action on the decay phase of I_{Ca} at the same concentration. Long-acting barbiturates such as phenobarbitone and barbitone are less lipophilic than ultra-short- and short-acting barbiturates such as thiopentone and pentobarbitone, as determined by their partition coefficients between methylene chloride, a typical lipid solvent, and water (Bush, 1963). Barbiturates may cause a conformational change of the lipid milieu surrounding the calcium channel by their lipophilic properties, resulting in an acceleration of the decay phase of I_{Ca}. This idea can be supported by the following observations: (1) Organic solvents such as *n*-alcohols also accelerated the decay phase of I_{Ca}, and double-pulse experiments on the inactivation showed the increased rate of voltage-dependent inactivation of I_{Ca} in the presence of *n*-alcohols. Concentrations of *n*-alcohols required for an obvious acceleration of the decay phase of I_{Ca} decreased as the hydrocarbon chain of *n*-alcohols increased in length (Oyama, Nishi & Akaike, 1983b). Increases in the length of the hydrocarbon chain of *n*-alcohols decrease the degree of hydrophilic-lipophilic balance (HLB), and alcohols are well-known to perturb the structure of the lipid bilayer membrane. (2) Organic calcium antagonists caused a slight acceleration of the decay phase of I_{Ca} during depolarizing pulses lasting for 4–8 s, while inorganic calcium antagonists such as divalent metal cations, which are not lipophilic, did not accelerate the decay phase of I_{Ca} (Oyama, Nishi & Akaike, 1983c). A local anaesthetic, such as lignocaine, also accelerated slightly the decay phase of I_{Ca} during long depolarizing pulses (Oyama & Nishi, unpublished observations). Thus, other mechanisms to explain the accelerating action of pentobarbitone on the decay phase of I_{Ca} as discussed in the previous paper (see Nishi & Oyama, 1983) were unlikely. Therefore, we conclude that barbiturates accelerate nonspecifically the decay phase of I_{Ca} by increasing the rate of the voltage-dependent inactivation which would be induced by conformational changes in the lipid milieu surrounding the calcium channel.

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