Influence de la nystatine sur la croissance, la conidiation et la synthèse des stérols libres et estérifiés de la souche sauvage et du mutant (4 jours de croissance en milieu liquide)

Souche	Source d'azote	Nystatine (ppm)	Poids sec mycélium (mg/l)	Nombre de conidies par g de mycélium $\times 10^7$	Stérols libres et estérifiés (mg/g mycélium)
Sauvage	Phenylalanine	0	409 ± 22	–	8,4 ± 0,8
Sauvage	Phenylalanine	2	276 ± 2	–	7,6 ± 0,3
Sauvage	Arginine	0	705 ± 107	6,6 ± 3,7	11,6 ± 0,9
Sauvage	Arginine	2	521 ± 28	0,4 ± 0,9	7,8 ± 1,3
Mutant	Phenylalanine	0	375 ± 13	–	11,8 ± 0,5
Mutant	Phenylalanine	2	287 ± 13	–	11,8 ± 0,2
Mutant	Argine	0	784 ± 50	8,7 ± 6,6	15,7 ± 1,9
Mutant	Arginine	2	840 ± 150	48,7 ± 18,6	14,2 ± 0,6

est, par contre, dépourvu de ces stérols liés par complexes; il compense partiellement ce manque par une teneur plus élevée en stérols libres et estérifiés. La possibilité de sélectionner de tels mutants devrait permettre d'éclaircir le rôle des stérols liés par complexes dans les cellules végétales; on admet généralement qu'ils constituent une réserve de stérols facilement disponibles[14].

La nystatine influence relativement peu la synthèse des stérols liés par complexes chez la souche sauvage. Cet antibiotique induit par contre abondamment la synthèse de cette catégorie de stérols chez le mutant. Cette augmentation de la teneur en stérols liés par complexes peut constituer un mécanisme de défense de la cellule contre la nystatine. Un tel mécanisme a été invoqué pour expliquer l'augmentation de la synthèse des stérols libres et estérifiés sous l'effet de la nystatine[15] et de la sulfanilamide[16] chez les levures. Les 2 souches du coprin ont le même spectre qualitatif de stérols; celui-ci contient très probablement de l'ergostérol et du 22-dihydroergostérol. Il n'est pas influencé par la nystatine.

La nystatine change non seulement la synthèse des stérols mais encore l'intensité de la conidiation; celle-ci diminue chez la souche sauvage en présence de l'antibiotique et augmente par contre fortement chez le mutant. Dans le milieu à base de phénylalanine, la nystatine n'arrive pas à induire la formation de conidies. Une fois de plus, les changements stéroliques provoqués soit par des mutations soit par l'addition de stérols exogènes[17] sont accompagnés de variations dans l'intensité de la reproduction asexuelle; ces variations sont beaucoup plus marquées que celles de la croissance mycélienne.

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Ba^{++} induced square-shaped potential waves in molluscan neurones

M. Gola and C. Ducreux

Département de Biophysique des Neuromembranes, C. N. R. S., Institut de Neurophysiologie et Psychophysiologie, 31, Chemin Joseph-Aiguier, F–13274 Marseille Cedex 2 (France), 2 Septembre 1976

Summary. Rhythmic slow square-shaped potential waves lasting 20–50 sec are induced in Aplysia neurones when Ca^{++} is replaced by Ba^{++}. During the plateau, the membrane is highly permeable to Ba^{++} ions, whereas in the inter-wave period the membrane resistance increases by 60 times. Analysis with slow current ramps suggests that the membrane properties of Ba-treated neurones and normally burst-generating neurones are similar.

During the last few years, much attention has been paid to the membrane mechanisms generating the slow potential oscillations in molluscan burst-generating neurones and in neurones exhibiting paroxysmal depolarization shifts (PDS). Our present ideas about these mechanisms are derived mainly from experiments performed in normal or pentylenetetrazol-treated neurones of molluscs. Besides, it was reported that Ba^{++} induces 'square shaped' slow waves in various nerve cells [1–4]. The analogy between these different electrical slow behaviours makes the Ba effect particularly interesting, since it can be easily reproduced. The present communication deals with the slow waves induced by replacing Ca^{++} by Ba^{++} in Helix and Aplysia neurones.

The experiments were performed on Helix pomatia and on a small specimen (5–10 g) of Aplysia rosea collected along the Mediterranean coast. The abdominal ganglion was removed from the animal and 2 KCl-filled electrodes were inserted in the cell. The Aplysia neurones studied were R_{14}, R_{15}, R_2 to R_4 and L_{11}. The most constant effect of Ba^{++}-saline was observed on R_{14} and L_{11}. All following results refer to R_{14}. Voltage measurements were made with conventional methods.

After control tests of the membrane characteristics of the cell, the artificial sea water (ASW) was changed to ASW in which Ca^{++} was replaced by Ba^{++}. In order to avoid a precipitate of $BaSO_4$, the $MgSO_4$ (20 mM) of the normal ASW was replaced by an isosmotic amount of $MgCl_2$ (NaCl 494 mM, KCl 10 mM, $MgCl_2$ 50 mM, Tris pH 7.7

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10 mM, $BaCl_2$ 10 mM.) Immediately after perfusion with the Ba^{++}-ASW, the cell which is normally silent begins to fire regularly. Then the spikes tend to group in bursts of a few spikes (figure, $A:A_1$). This period of hyperexcitability is followed by a silent period during which the cell hyperpolarizes (2–5 mV). The slow waves appear from the hyperpolarized state, 15–20 min after Ba application and after 30 min, their frequency and shape are constant. In the inter-wave period, the potential is close to –40 mV (figure $A:A_2$).

The slow waves are preceded by a slow depolarization (3 mV/min) which triggered a burst of spikes of increasing duration. Finally, a slow wave appears on the repolarizing phase of the last spike of the burst. This pattern reproduces regularly for several h with a 4 min period. The duration of the slow wave and of the inter-wave period can be increased or decreased by inward or outward current injection. The slow depolarization preceding the burst of spikes turns to slow hyperpolarization under moderate (5 nA) inward current injection, indicating a reversal potential close to –60 mV. The plateau level of the wave is between +20 and +30 mV and lasts 35 sec (20–50 sec). It varies with the concentration of the Ba^{++}: between 2.5 and 20 mM Ba^{++}, the plateau level is linearly related to the logarithm of the Ba^{++} concentration with a slope of 29 mV per decade corresponding to the theoretical value for a pure Ba^{++} electrode.

The slope resistance of the membrane, tested by short current pulses (500 msec, 1–5 nA) undergoes cyclic variations: it is maximal just before the spiking (6 MΩ) and reduces to 0.1 MΩ at the beginning of the plateau (figure, B). During the slow decaying phase of the plateau, and during the slow depolarization in the inter-wave period, the membrane resistance increases. Similar results were obtained on Planorbis neurones bathed in Ba-containing saline[3].

It can be concluded that on the depolarized plateau, the membrane becomes highly permeable to Ba ions. It is to be noted that adding of 10 mM Ca^{++} or 10 mM Co^{++} to the Ba-ASW blocks the slow waves whereas TTX (up to 5×10^{-5} M) has no effect. The current-voltage relation of the membrane was determined during a current clamp.

We used a slowly linearly increasing current (slope ≃ 1 nA/sec) followed with a variable delay by a symmetrically decreasing current (figure, C). Direct X–Y plots of the current-voltage relationships are shown in the figure, D. In both directions, the i-V curves display a flat region corresponding apparently to a very low conductance state, but the current value of that flattened region depends on both sign of current ramp and initial conditions. With a short delay (0–20 sec) between ramp pulses, the potential trajectory in the i-V plane describes a clockwise hysteresis loop (figure, $D:D_1$), i.e. for corresponding current levels, the potential is more negative with the repolarizing ramp than with the depolarizing one. With a longer delay (figure, $D:D_2$) (more than 25–30 sec) the hysteresis reverses to a counterclockwise loop. This behaviour, already observed in PTZ-induced burst-generating[5] and in warmed L_{11} Aplysia neurones[6], can be interpreted as the effects of a quasi steady-state negative resistance whose magnitude and position in the i-V plane depend on the initial state of the membrane. Such a potential-dependent negative resistance is an essential characteristic of normally burst-generating neurones[4, 7, 8]. It thus appears that the various slow potential waves are produced by similar ionic mechanisms. It can be hoped that an extensive analysis in voltage clamp conditions of Ba-treated neurones would give data, (for the moment still partial) necessary to reconstruct the slow potential behaviour of pacemaker neurones.

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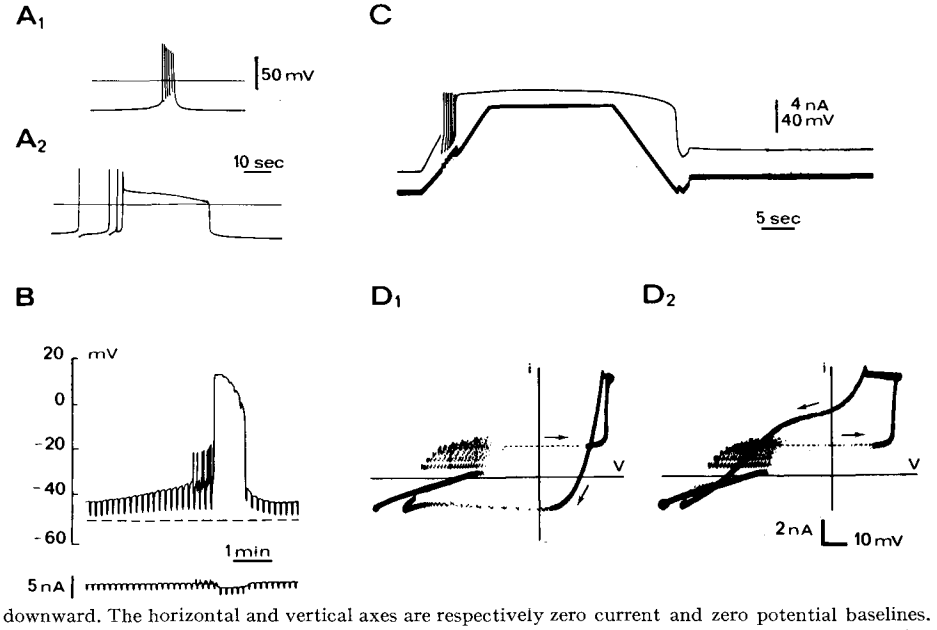

Effects of substitution of Ca^{++} by Ba^{++} on R_{14} neurone of Aplysia. *A* Spontaneous burst of spikes and square-shaped slow wave obtained respectively 5 min (A_1) and 25 min (A_2) after Ca was replaced with Ba^{++}. The zero potential level is indicated by the horizontal line. *B* Pen-recording of potential changes (ΔV) induced by regular inward current pulses ΔI. The slope conductance $\Delta I/\Delta V$ is greatest at the beginning of the slow wave and declines during the plateau and in the interwave interval (action potentials are reduced by the time constant of the pen recorder). *C* Potential changes (upper trace) induced by depolarizing and then repolarizing current clamp ramps (1 nA/sec) (lower trace). *D* Direct display of the current-voltage relationship determined with the procedure described in C; in D_1 and D_2, the 2 ramps are separated by 18 and 42 sec respectively. Inward currents are downward. The horizontal and vertical axes are respectively zero current and zero potential baselines. Arrows show the directions of the trace starting from the left. The dashed lines outline the rapid depolarizing phase of the last spike just preceding the plateau. The hysteresis loop is described clockwise in D_1 and counterclockwise in D_2.