

EFFECT OF CALCIUM ON TOAD SKIN POTENTIALS

Efectos del calcio sobre los potenciales de piel de sapo.

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RESUMEN

Dos potenciales de acción Calcio-dependientes se pueden evocar cuando se pasan a través de la piel aislada de sapo (*Pleurodema*) pulsos estimulantes de polaridad apropiada y 300 milisegundos de duración; un potencial rápido (3-8 mseg. de duración) y un potencial lento (50-80 mseg. de duración). El primero puede evocarse, en la ausencia del último, cuando las pieles se estimulan con pulsos de menor duración (35 mseg.). Un aumento del Calcio externo resulta en un aumento de la resistencia de la piel y una disminución del umbral de estimulación del potencial rápido, a la inversa, en ausencia de Calcio no se aprecian variaciones significativas de la resistencia. Cuando el medio externo se hizo hipertónico, sólo se evocó el potencial lento y no el rápido. Se sugiere que la génesis del potencial rápido es debido a la movilización de Calcio por la corriente estimulante, el cual a su vez disminuye la permeabilidad al Sodio, a través del paso "shunt" del epitelio de la piel de sapo con el consiguiente aumento en resistencia. Por otra parte, la génesis del potencial lento es diferente ya que puede ser abolido por inhibidores metabólicos.

ABSTRACT

Two calcium-dependent action potentials can be evoked when pulses of proper polarity and 300 msec of duration are passed across isolated toad skins (*Pleurodema thaul*): a Fast potential (3-8 msec duration) and a Slow potential (50-80 msec duration). The former can be elicited, in the absence of the latter, when the skins are stimulated with pulses of shorter duration (35 msec). An increase in the external calcium resulted in an increased skin resistance and lowering of the stimulating threshold of the Fast potential, conversely, in the absence of calcium no significant resistance variations were seen. When the external medium was made hypertonic, the Slow, but not the Fast potential could be evoked. It is suggested that the genesis of the Fast potential is due to the mobilization of calcium by the stimulating current, which in turn decreases the permeability of sodium across the shunt path of the toad skin epithelium with a consequent increase in resistance. On the other hand, the genesis of the Slow potential is different since it can be abolished by metabolic inhibitors.

Keywords: Amphibia. Calcium activity. Physiology.

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INTRODUCCION

Frog and toad skins have the property of transporting electrolytes against electrochemical potential gradients and have been extensively used as a model for the study of biological membranes. This charge transfer gives rise to a significant potential difference across the skin, which is maintained for several hours and can be affected by certain hormones and pharmacological agents (Koefoed-Johnsen, Ussing and Zerahn, 1953; González, Sánchez and Concha, 1966, 1967).

Finkelstein (1961, 1964) found that frog skins were electrically excitable when stimulated by a current of proper polarity and sufficient intensity and showed that this phenomena could be modified by changing the ionic composition of the solution bathing the outer, but not the inner surface of the skin. The effects of some ions and possible response sites were discussed and the similarities of this action potential and those of nerve and muscle were analyzed.

In this work an attempt is made to explain the action potentials of the toad skin in ionic terms and a role for calcium is postulated.

MATERIALS AND METHODS

All experiments were performed "in vitro" on the abdominal skins of both male and female toads of the species *Pleurodema thaul*. No seasonal variations were observed. The skins, removed from pithed toads were stretched out and mounted between two compartments of a lucite chamber which were bathed with 3 ml of toad-Ringer solution (NaCl 122 mM; KCl 1.9 mM; CaCl₂ 2 mM; NaHCO₃ 2.3 mM; Glucose 11 mM) bubbled with oxygen and buffered to pH 7.4. The exposed surface of the skin was 2 cm².

A pair of platinum stimulating electrodes, connected to an isolation unit of a Grass model S4C Stimulator, were used for passing pulses of current across the preparation. The potential variations were measured with a Tektronix 5112 Oscilloscope through a pair of calomel electrodes, which were connected to the solution bathing both sides of the skin through agar-Ringer bridges (fig. 1, A).

The intensity of the current was calculated from the potential generated by the stimulating current between the two ends of a fixed resistance (180 ohms) placed in the circuit (fig. 1, A).

In some experiments the toad-Ringer solution bathing the outer surface of the skin was replaced by NaCl (154 mM) containing 1.1 or 3.3 mM CaCl₂.

A waiting period of 30 to 50 min was allowed after mounting in order to obtain a stabilized resting potential. Stimulations were performed every .90 sec in order to allow for the 5 to 20 sec refractory period.

RESULTS

A. *Types of Potentials.* Two types of action potentials could be elicited when pulses of proper voltage and polarity, of 300 msec duration, were applied to the skin: a Fast potential of short duration (3–8 msec) and a longer lasting (50–80 msec) and lower voltage, Slow potential (fig. 1 B). The two could be studied separately since only the fast potential was evoked when short stimulating (35 msec) currents were applied.

B. *Effect of Calcium on the Fast Potential.* As shown in fig. 2, the Fast potential could not be elicited when the toad-Ringer solution bathing the outer surface of the skin was replaced by 154 mM NaCl. However, it could be evoked when 1.1 mM CaCl_2 was added to the saline.

In fig. 3, A, are shown the current-voltage curves obtained when two different calcium concentrations were tested. In both it can be seen that, when the threshold of the action potential was reached, abrupt resistance changes and rectification phenomena were produced. However, the higher calcium solution gave a greater resistance change and the action potential was produced at a lower threshold. When the outer medium was replaced by a calcium-free solution, containing 154 mM NaCl and 1.1 mM Glucose, neither the rectification phenomena nor the abrupt resistance increase were observed (fig. 3, B).

Fig. 4 illustrates the intensity of the applied stimulus (volts) against the calculated resistance. Again it can be observed that the higher the calcium concentration, in the solution bathing the outer surface, the lower the firing threshold of the action potential and the greater the resistance increase. Conversely, in the absence of calcium, no significant resistance variations were seen as stimulus intensity (volts) was increased.

C. *Effect of Calcium on the Slow Potential.* As was the case for the Fast potential, the Slow potential could not be evoked unless the solution bathing the outer surface of the skin was toad-Ringer (fig. 5, a) or 154 mM NaCl containing 1.1 mM CaCl_2 (fig. 5, b). When a calcium-free solution was used neither potential was observed (fig. 5, c).

On the other hand, when the skins were mounted immediately after removal from the animal and bathed with a calcium-free external medium, the Slow potential could be evoked, but it decreased with time (fig. 6). The addition of 1.1 mM CaCl_2 to the outer solution elicited an immediate appearance and restoration of the Fast and Slow potentials respectively.

The Slow potential could be independently abolished by the addition of 20 mM EDTA to the solution bathing the inner surface of the skins. On the other hand, Tetrodotoxin (10^{-7} M) added to the external solution, affected neither the resting nor the action potentials of the preparation.

The Fast potential could not be elicited when the external solution was made hypertonic (toad-Ringer + 300 mM sucrose). On the other hand, the Slow potential was only slightly altered under these conditions. Conversely, the addition of 2-4 dinitrophenol (0.5 mM) or NaCN (2 mM) to the outer solution caused a complete inhibition of the Slow potential without affecting the Fast potential.

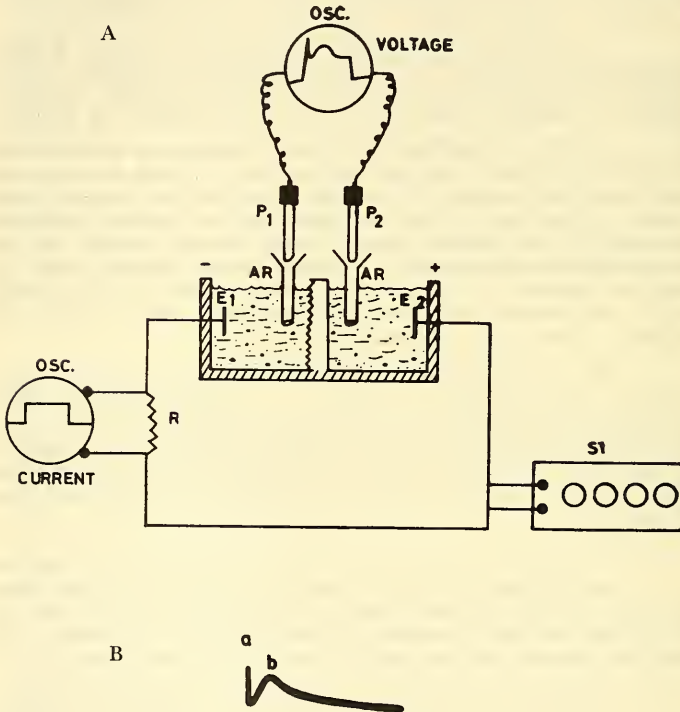


Fig. 1.- A) Diagram showing toad skin chamber and electrical circuits: S: toad skin; P₁ and P₂: calomel electrodes; AR: agar Ringer bridges; E₁ and E₂: stimulating electrodes; St: Stimulator; Osc: Oscilloscope; R: 180 ohm resistance.

B) Typical action potentials across isolated toad skin, in response to a stimulus of 20 volts and 300 msec duration. a: Fast Potential, b: Slow Potential.

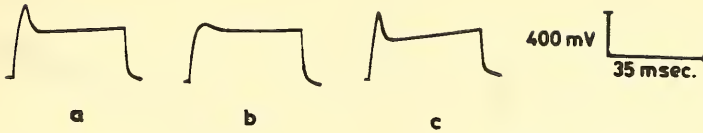


Fig. 2.- Records that show the effect of external calcium on the Fast potential. The external medium were: a) Toad Ringer solution; b) 154 mM NaCl and c) 154 mM NaCl + 1.1. mM CaCl_2 . The skins were stimulated with pulses of 13 volts and 35 msec duration.

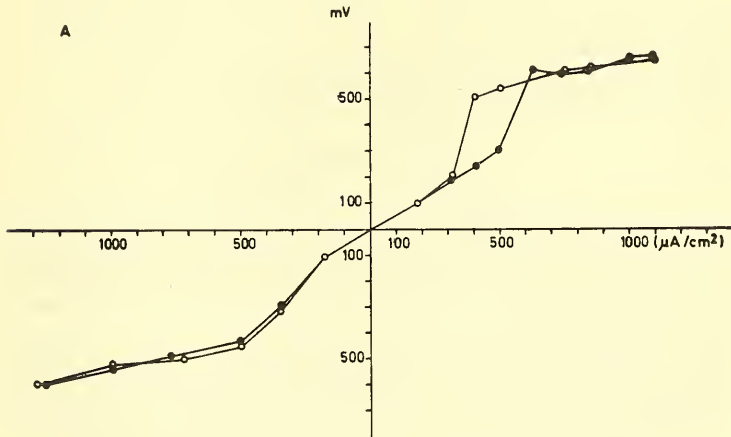


Fig. 3.- Current-voltage curves obtained with 154 mM NaCl solution.

A) in the presence of calcium, (●) 1.1. mM and (○) 3.3. mM calcium. The abrupt rise in voltage indicates the occurrence of the action potential.

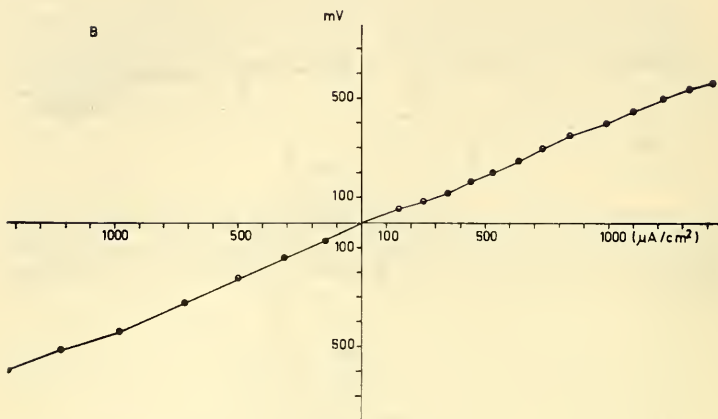


Fig. 3.- (Continued).

B) in the absence of calcium but with 1.1 mM glucose. (0)

DISCUSSION

Our results show that two different types of action potentials could be generated in the skin of the toad *Pleurodema thaul* when stimulated with quadratic currents of 300 msec duration and that there was no relation between the magnitude of these potentials and the resting potential of the skin.

The first, or Fast potential, has a duration of 5 to 8 msec and the second, or Slow potential, was of a lower millivoltage and was longer lasting (50–80 msec). Fig. 2 shows that when the skins were stimulated with shorter pulses (35 msec) only the Fast potential could be evoked, a fact, which allowed us to study both in a differential manner.

Fig. 4 demonstrates that there was an abrupt calcium-dependent resistance increase in the skin when the Fast potential was produced which was not observed when calcium-free solutions were used. Conversely, as the calcium concentration was increased, the voltage necessary to reach the firing threshold was lower and an increase in the resistance of the skin was seen. As is later discussed, the resistance change is likely to be caused by an abrupt decrease in the sodium flux across the skin.

Ussing and Windhager (1964) have demonstrated that sodium movements across frog skin epithelia follow two pathways, an active transport path and a shunt pathway for the passive movements of sodium as well as other ions. In our experiments the Fast potential was unaffected

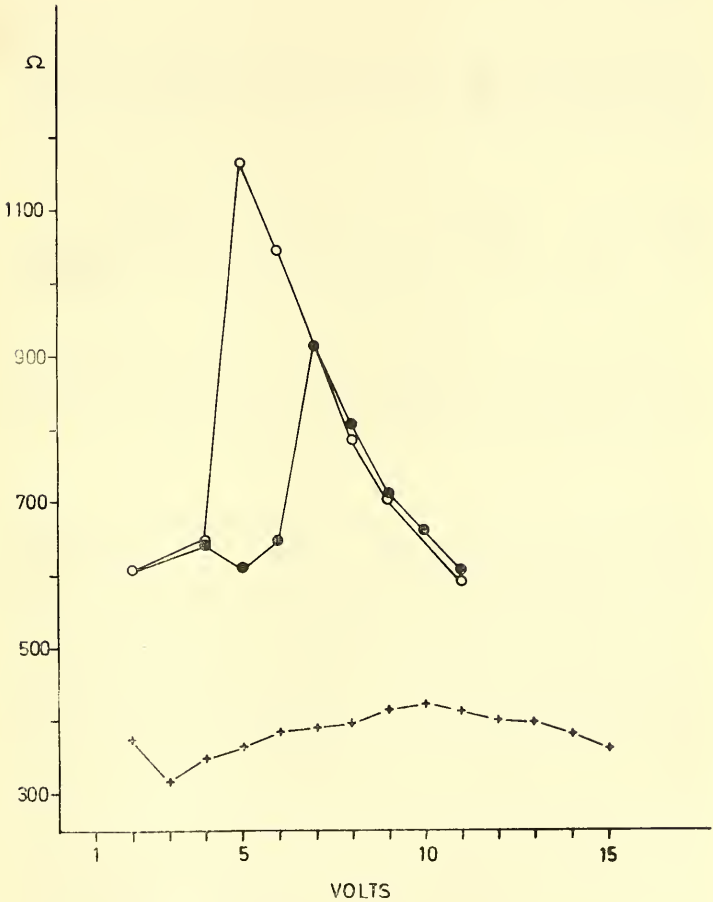


Fig. 4.- Relationship between the intensity of the applied stimulus (volts) and the calculated resistance. (●) 154 mM + 1.1. mM CaCl₂; (○) 154 mM NaCl + 3.3. mM CaCl₂ and (X) 154 mM NaCl + 1.1 mM glucose.

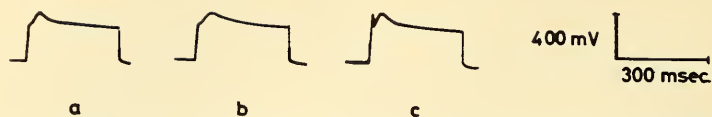


Fig. 5.- Records that show the effect of external calcium on the Slow potential. The external medium were: a) Toad Ringer solution; b) 154 mM NaCl + 1.1 mM CaCl_2 and c) 154 mM NaCl + 1.1 mM glucose. The skins were stimulated with pulses of 20 volts of 300 msec duration.

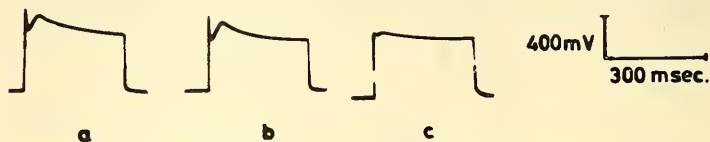


Fig. 6.- Effect of added external calcium on the Fast and Slow potentials, when the skin was mounted and bathed externally with 154 mM NaCl. The skins were stimulated (20 volts, 300 msec) at the following times after mounting: a) 1 min, and b) 10 min. The effect of added calcium can be seen in c.

when the skins were treated with NaCN (2 mM) or 2-4 dinitrophenol (0.5 mM) in the outer bathing solutions, but the Slow potential disappeared suggesting that the genesis of the former is independent of cellular metabolism and might be produced by a hindrance, by calcium, of the passive movement of sodium through the shunt pathway.

Other authors (Urakabe, Handler and Orloff, 1970; DiBonna, 1972; DiBonna and Civan, 1972, 1973; Wade, Revel and DiScala, 1973; Civan and DiBonna, 1974, 1978) have demonstrated that the shunt conductance of the tight junctions of the toad bladder increases when the outside is made hypertonic with permeable solutes. In preliminary experiments we have observed that, when the outer surface of the skin is bathed with an hypertonic solution (toad-Ringer + sucrose 300 mM), the Fast potential disappears and no resistance changes can be observed. These results might be due to a probable opening of the tight junctions with a subsequent exaggerated increase in skin conductance. These results agree with those of Erlij and Martínez-Palomo (1972) who have shown that the tight junctions of frog skin can be reversibly opened when the medium bathing the outer surface is made hypertonic.

The link between calcium and sodium permeability is a rather well-established phenomenon in a number of excitable systems (Frankenhaeuser and Hodgkin, 1957; Adelman and Dalton, 1960; Dumont, Curran and Solomon, 1960). As indicated, an alteration in the Fast potential threshold was produced when the calcium concentration was varied in the outer medium (fig. 4). This result agrees with those of Curran

and Gill (1962) who demonstrated that variations in the calcium concentration in the outside bathing solution affected the short-circuit current and ionic movements in the frog skin, indicating, that calcium had a role in the regulation of the permeability of the outermost layers of the skin. It was also shown that changes in the calcium concentration of the inside bathing solution did not produce consistent or sustained effects on the properties of the preparation.

Mandel and Curran (1972) examined the properties of the shunt pathway (a pathway in parallel to the sodium transport system) in frog skins and showed that the permeability of this shunt increased at depolarizing potentials, was dependent on the external solute concentration and was considerably reduced by the presence of external calcium. The authors suggested that calcium might hinder the movement of sodium in the shunt. According to the above, we believe that the Fast potential of the skin is due to the mobilization of calcium in the outer solution, which in turn hinders the movement of sodium through the shunt pathway. As a consequence, a decrease in the conductance of this ion would be responsible for the observed resistance increase which gives rise to the Fast action potential.

The Slow potential is also a calcium-dependent phenomenon (fig. 5, b) but its mechanism and generation sites are different. When the skin was mounted immediately and bathed with a calcium-free solution in the external compartment, only the Slow potential could be evoked (fig. 6) but its magnitude decreased with time. The presence of the Slow potential, when the outer solution was calcium-free, is probably due to the movement of calcium from the inner deposits of the skin described by Hayashi, Takada and Watabe (1977) by the action of the stimulating current. Lindemann and Thorns (1967), showed that the Slow potential was absent from many of the microelectrode recordings at the outermost border of the frog skin, indicating that the probable site of the genesis of the Slow potential is located at a deeper level of the epithelium.

The Slow potential could be abolished by metabolic inhibitors indicating that it might be related to the active transport of sodium through the transport path described by Ussing and Windhager (1964).

A better understanding of the Slow potential should be obtained from experiments currently in progress.

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