Calcium transients in a crustacean motoneuron soma: Detection with arsenazo III

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Summary. Long lasting action potentials can be triggered in crayfish giant motor neurons by a short depolarizing pulse under different conditions. The concomitant increase in absorbance of the Ca indicator arsenazo III preloaded into the soma, confirms previous observations suggesting that these potential changes are related to a Ca inward current through the soma membrane.

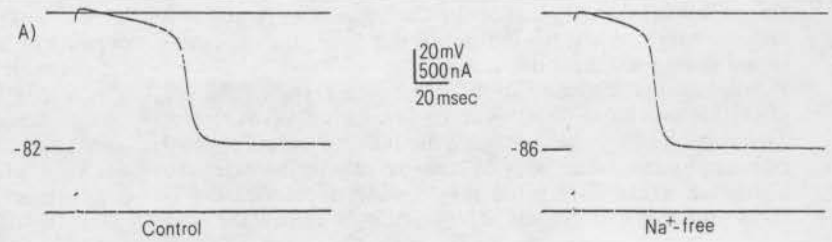
The giant motoneurone (MoG) is a large cell, found bilaterally in the crayfish abdominal ganglia. Early work[1] suggested that the cell soma does not show any regenerative action potential in response to a depolarizing pulse, but it is now known that regenerative responses can be recorded under certain conditions. For instance, when the K^+ conductance of the membrane is reduced (either by TEA[8] or following inactivation by a conditioning depolarisation), a slow action potential (SAP) may be triggered by a short depolarization. This response is most probably due to an influx of Ca^{2+} ions, since it depends upon the presence of Ca^{2+}, but not Na^+ in the bathing solution, and is blocked by Co^{2+} and Mn^{2+}, but not by TTX[2]. In the preparations pretreated with TEA, the SAPs can also be recorded at more hyperpolarized levels than the normal resting potential. TEA is effective either injected into the MoG soma or added to the saline[3]. In this paper most of the results were obtained in this way when TEA was added to the solution.

We report below that the internal free Ca concentration increases during the SAP, as demonstrated by the change in absorbance of the Ca^{2+} indicator dye arsenazo III preloaded into the MoG soma. This observation strengthens previous observations suggesting that SAP is associated with an influx of Ca^{2+} ions.

Methods. Experiments were carried out on the giant motor neurons of the crayfish *Procambarus clarkii*. The ventral chord was dissected and perfused with Van Harreveld's solution containing (in mM): NaCl 195; KCl 5.4; $CaCl_2$ 13; $MgCl_2$ 2.6; Tris maleate 10, adjusted to pH 7.2 by NaOH. The 2nd, 3rd and 4th ganglia were desheathed and used for the experiments. The membrane potential was monitored by conventional electrophysiological techniques using a 3 M KCl electrode. A 2nd electrode filled with an aqueous solution of 1 mM arsenazo III (Sigma grade 1) was also inserted in the MoG soma. The dye was injected by negative current pulses (500 msec, 1 Hz) which hyperpolarized the cell by 30–40 mV. The injection was stopped when the soma appeared just detectably stained as judged by eye. The Arsenazo electrode was then used for passing both the conditioning depolarizing current pulse and the brief (2–5 msec) pulse triggering the SAP. The intracellular calcium transients were monitored by recording the differential absorbance of the dye at 650 and 700 nm as previously described[4,5].

Results. Typical responses obtained in TEA-treated preparations are shown in figure 1. The SAP was almost unchanged in Na-free solutions (fig. 1A). On the contrary, when Ca^{++} was omitted from the bathing solution, the SAP was abolished (fig. 1B). These observations, together with the fact that Co^{2+} and Mn^{2+}, but not TTX, block the SAP make it very likely that the response is due to an influx of Ca^{2+} ions[2,3]. It is therefore interesting to look for possible changes in intracellular ($[Ca^{2+}]_i$) during the SAP.

Simultaneous records of membrane potential and arsenazo absorbance from a MoG cell soma are shown in figure 2. Following a conditioning depolarization from the resting potential ($V_r = 66.9$ mV ± 5.3; $N = 59$), a long lasting action potential is elicited by a brief depolarizing pulse in preparations not treated with TEA (fig. 2A). During the SAP a rise in arsenazo absorbance is seen, which returns slowly to the baseline after the cell has repolarized. This indicates that the intracellular free Ca^{2+} level increases steadily throughout the duration of the SAP. Control records obtained at a wavelength of 570 nm (the isosbestic point of the dye)[5] demonstrated that the arsenazo Ca^{2+} signal is not appreciably contaminated by any other optical changes



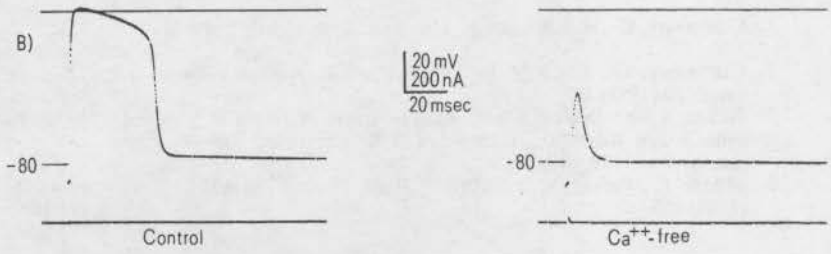


Figure 1. Effects of Na^+ and Ca^{2+} removal upon the SAPs triggered at the resting potential in MoG pretreated for at least 30 min in solutions containing 75 mM TEA. *A* Before (left) and after (right) substitution of Na^+ by an equimolar concentration of choline. *B* Before (left) and after (right) total substitution of Ca^{2+} by an equimolar concentration of Mg^{2+}. Top trace: intracellular potential; bottom trace: triggering current.

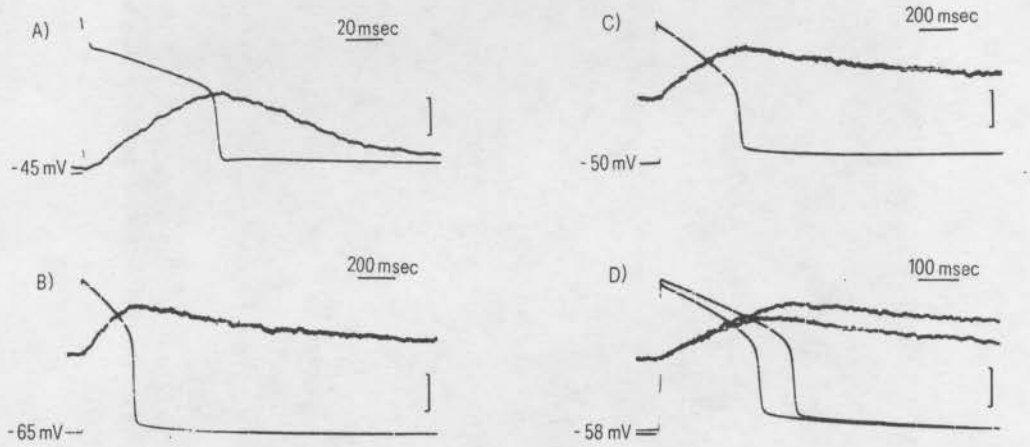


Figure 2. Arsenazo light responses (upper trace) and slow action potentials (lower trace) recorded in different conditions. The optical record is a differential recording of light transmission at the wavelength pair 650-700 nm. An upward deflection corresponds to an increase in dye absorbance at 650 nm, and signals an increase in free (Ca^{2+}). The starting point of the arsenazo trace was reset at the beginning of each sweep. *A* In normal saline, the soma potential was shifted from the resting potential to -45 mV about 5 sec before applying the triggering pulse. *B, C, D* Responses of another cell in the presence of 75 mM TEA. Single depolarizing pulses applied at holding potentials of -65 mV (B) and -50 mV (C) triggered action potentials of different duration. *D* Two depolarizing pulses were applied 2 sec apart at a holding potential of -58 mV. In response to the 2nd pulse, the duration of both signals was decreased.
Calibrations: Membrane potential = 20 mV, arsenazo signal expressed as $\Delta I = 1.7 \times 10^{-3}$ (A) and 3.6×10^{-3} (B, C, D).

during the SAP. In a saline containing 75 mM TEA, a similar transient increase in absorbance occurred during the SAP (fig. 2B).

The relationship between the duration of the SAP and that of the rising phase of the arsenazo signal could be directly verified by two different methods using the same cell: 1. The SAP duration strongly depends on the holding membrane potential. When the latter was shifted from -65 mV (fig. 2B) to -50 mV (fig. 2C), both the duration of the SAP and the rising phase of the arsenazo signal increased by about 50%. However, the rate of rise of the arsenazo signal then decreased, the amplitude being similar to that observed at -65 mV. 2. The SAP duration (and to a much lesser extent its amplitude) is very sensitive to repetitive activation[2]. Two short depolarizing pulses applied 2 sec apart to the same cell triggered SAPs of decreasing duration (fig. 2D) though the 2nd SAP had been triggered at a slightly more depolarized level. Again the duration of rise of arsenazo signal fitted that of the SAP.

Unlike the other motoneurons, MoG does not arborize in the neuropile. Its neurite is very thin at the beginning and leaves the soma obliquely in a dorso-caudal direction[6,7]. The area analyzed by the optical system could thus be limited by an image of a circular diaphragm focussed onto the larger part of the soma. This minimized a possible contribution of other parts of the cell to the recorded arsenazo signal.

This gives additional evidence that the SAP results from an inward Ca current through the soma membrane. Quantitative measurements of $[Ca^{2+}]_i$ are now under investigation. The records in figure 2 show a steady increase in $[Ca^{2+}]_i$, which suggests that most of the Ca^{2+} signal is due to a

Experientia 40 (1984), Birkhäuser Verlag, CH-4010 Basel/Switzerland

steady inward flux. If most of the Ca^{2+} signal were due to a sudden surge at the beginning of the SAP, the arsenazo signal should not show this steady rise.

Furthermore, the time constant of the membrane ($\cong 4$ msec at rest) is much too small to account for the SAP duration. Since the SAP is still observed in Na-free solutions and requires external Ca^{2+} (fig. 1), the increase in the arsenazo signal occurring during the long lasting depolarization is most probably due to the Ca^{2+} entering the cell rather than to Ca^{2+} released from cytoplasmic pools secondary to depolarization. Thus a more precise characterization of the Ca channels and of the mechanisms underlying their inactivation may be expected from the use of the arsenazo dye. The kinetics of Ca^{2+} entry and Ca^{2+} removal obtained from the arsenazo signal will be especially useful for studying whether internal Ca^{2+} concentration affects Ca conductance on this preparation as it does in other preparations[9,11].

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0014-4754/84/010106-03$1.50 + 0.20/0