BULLETIN OF TAIPEI MEDICAL COLLEGE, VOL. 9, Oct. 1978
p.3-15

Effect of Changes in Some Ions Concentration on the Spontaneous Electrical Activity of the Marine Lobster Cardiac Ganglion

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The effects of change in the external K and Ca concentration on the electrical activity of the cardiac ganglion was intracellularly studied in the Japanese spiny lobster, (panulirus japonicus).

Results may be summarized as follows:

1. *The resting potential of the active soma was between 33-65mv, but may of them about 50 to 55mv. The amplitudes of the maximal potential and of the sustained potential in the burst of the soma increased as the resting potential of the soma increased. The increment was larger in the former than in the latter.*
2. *The resting potential and sustained potential of the soma attained a steady value in few minutes when external K concentration was changed and in several minutes when external Ca concentration was changed.*
3. *The excess of K decreased the membrane potential, shortened the interval and duration of the burst, and decreased the amplitude of action potentials, high K often initiated the spontaneous spike during the inter burst, deficiency of K affected the burst activity in a reversed way.*
4. *The excess of Ca increased the membrane potential, lengthened the interval and duration of the burst, and increased the amplitude of action potentials, high Ca often depressed the activity of the cell. Deficiency of external Ca affected in a reversed way.*
5. *It was shown that the membrane potential of the soma was not accounted for well on the basis of change in the K potential alone from relation between the external K concentration and resting potential of the soma.*
6. *It was shown that the stabilizing effect of Ca was relatively small from relation between external Ca concentration and the resting potential of the soma.*
7. *Change in the burst interval per unit change in membrane potential was greater in Ca solution than in K solution.*
8. *Change in the amplitude of the sustained potential per unit change in membrane potential of the soma was in high Ca solution than in low K solution.*

Key words: *External K and Ca ions effects, Lobster cardiac ganglions, Spontaneous electrical activity, Panulirus japonicus.*

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Received for Publication: May 19, 1978

This investigation was undertaken to analyse the effects of external K and Ca concentrations on the ganglionic trunk was studied intracellularly to know some relations between the membrane potential of the large soma and the rhythmical burst activity. The ganglionic trunk of the crustacean heart discharges spontaneously with a regular rhythm corresponding to the heart best. The effect of changes in external cation on the rhythmical burst activity in the ganglionic trunk of the Japanese spiny lobster was studied extracellulary (Matsui). [13] The external K, Ca, Mg and Na were shown to have characteristic action on the interval and duration of the burst, on the frequency of spikes and on the slow wave. Recently intracellular potential changes in lobster cardiac ganglions have been studied by many authors. Usually the burst is consisted of series of synaptic potentials and spike potentials superimposed on them (Hagiwara and Bullock). [10] A long sustained depolarization characteristic of the burst is also observed in the typical activity (Bullock and Terzuolo) [3]

Materials and Methods

The material used in this study was the Japanese spiny lobster, (panulirus japonicus) which ranged from 200 to 300 gm. in body weight. The heart was isolated as described previously (Matsui) [13] Experimental procedure was similar to that described by Hagiwara and Bullock. [10] Occasionally the preparation was stained with methylene blue by the method described by Alexandrowicz. [2] The physiological salt solution used was composed of 100 parts of 0.6 M NaCl, 2 part of 0.6 MKC1, 5 parts of 0.4 M $CaCl_2$ and 7 parts of 0.4 M $MgCl_2$ solutions and the solution was adjusted to PH 7.3 by adding $NaHCO_3$, contents of K and Ca in this solution are similar to those in a marine lobster serum (Homarus, Cole), [4] while contents of Na and Mg are somewhat higher. Previous experiments (Matsui) [13] have shown that the ganglionic trunk of the Japanese lobster heart maintains the regular rhythm for hours in this solution.

Experimental solution of various K or Ca concentrations were prepared by adding more or less of respective solution. Amount to be added was changed ¼, ½, 2 and 4 times normal. These solution are respectively as referred ¼ Ca, 4 Ca, ¼ K, 4 K and so forth in this paper. K concentration ranged between 2, and 40 mM and Ca concentration between 4.5 and 62 mM.

Capillary microelectrode fille with 3 MKC1, of which electric resistance was 10-30 megaohm, was penetrated into the large soma of anterior cells by means of a micromanipulator under binoculars. The microelectrode was connected with a dual beam oscilloscope through an amplifier with an input cathode follower of grid current.

The experiments were carried out at room temperature which was between 15 and 25°C.

Anatomical Description of Marine Lobster Heart

The anatomical structure of the Lobster heart employed in this work resembed to that of panulirus japonicus reported by Matsui. [14]

The cardiac ganglionic trunk of the marine spiny lobster, panulirus japonicus,

is similar to those of other lobsters such as palinurus vulgaris (Alexandrowicz), [2] and punulirus argus (Welsh). [22] It is situated in the dorsal cardiac wall near to the innter surface. It does not bifurcate and contain five large and four small ganglion cells.

The large cells are situated in the anterior half of the trunk, usually four of these are about half way down the trunk (Fig. 1). The four small cells are situated in the posterior half of the trunk with more or less equal spacing.

The sizes of the ganglionic trunk and of the ganglion cells vary more or less in different specimens, generally they correspond to the size of the animals. The length of the trunk from its anterior edge to the posterior is about 15 mm and in the larger specimens up to about 20 mm with many branches being distributed from both the anterior and posterior parts to the myocardium. The site of the ganglion cells in the trunk was observed with use of methylen blue staining, the large cells measure about 170 x 130μ, and in the large specimens up to about 230 x 200μ; the small cells measure about 50 x 35μ. It is easier to expose the anterior part of the trunk than the posterior, as the latter lies more deeply in the cardiac muscles. The five follower neurous receive input from the pacemakers, from each other, and from fibers from the central nervous system. Some of which accelerate and others of which inhibit the heart rate.

Spontaneous Discharges of the Isolated Ganglionic Trunk

Most of the isolated ganglionic trunk showed spontaneous discharges immediately or within about ten minutes after isolations. A periodic burst of spikes was recorded most frequently (Fig. 2)

In some cases, continuous or irregular trains of sipkes, or those in company with the periodic were recorded. The period of the burst which varied considerably accoroling to both the experimental temperature & preparations, shortened more or less in the process of time in the same preparation. This period may correspond to that of the heart beat. [22, 23]

Results

Preparations were bathed with experimental solutions usually for several minutes and then returned to the normal solution. Such procedure was repeated with various experimental solutions of different ionic concentrations.

Time courses of changes in the membrane potential and in the sustained potential of burst recorded in the soma when preparations were exposed to the solutions of the excess of Ca or deficiency of K were shown in Fig. 3. It was seen that both resting and sustained potentials began to rise as soon as the preparations was bathed and reached to steady values, which were attained in several minutes in Ca treatment and in a few minutes in K. Time courses for recovery in the normal solutions were similar in each case. Thus, the change was reversible.

1. Effects of K and Ca on the membrane potential of the soma:

   Resting potentials of the active soma ranged between 33-65 mv, but many of them about 50-55mv. The value accords with those in other kinds of

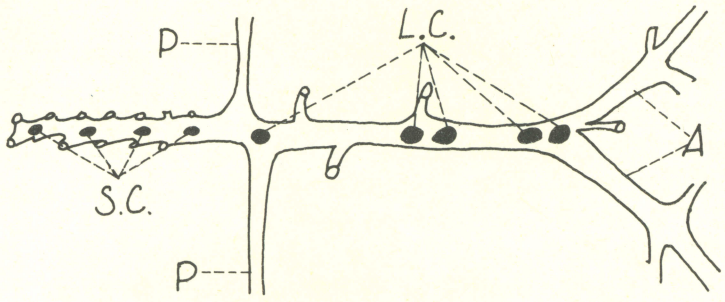

Fig. 1
Schematic sketch of cardiac ganglionic trunk of panulirus japonicus (inner surface of the heart wall view) L.C; Large ganglionic cells S. C; Small ganglionic cells A; Anterior part P; Posterior part.

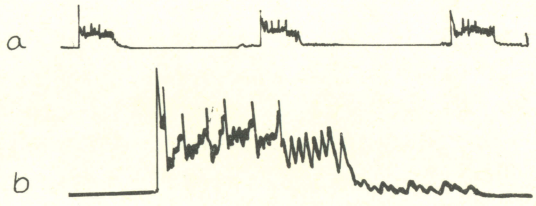

Fig. 2
Activity in the lobster cardiac ganglion. a & b show a single size of spike in the soma. Each heart best is represented by a burst of activity. a; on a slow time scale. b; enlarge of (a) burst. Calibration marks represent. 500 msec & 18 mv for (a), 110 msec & 4 mv for (b).

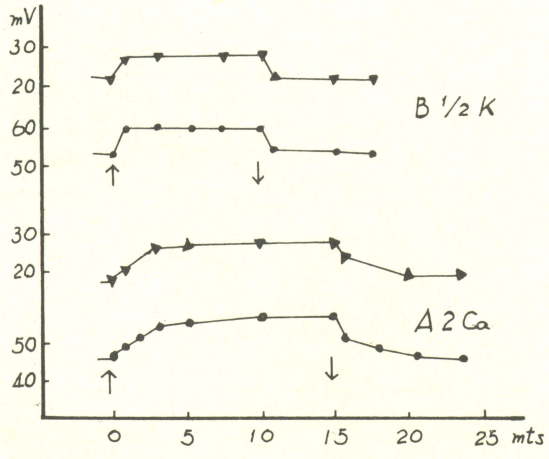

Fig. 3
Time course of potential changes in the large soma when external ionic concentrations were changed:

● : resting potential.
A : effect of 2 Ca.
▲ : sustained potential.
B : effect of ½ K.

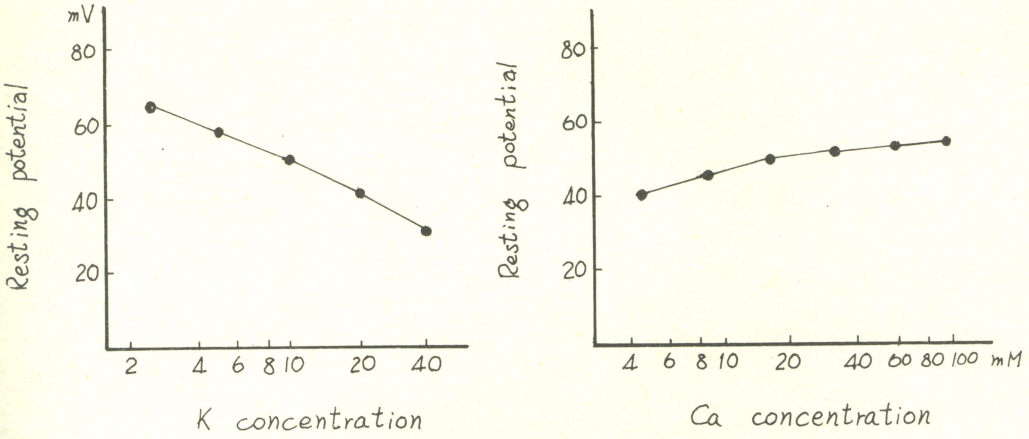

Fig. 4
Effects of external Ca & K concentrations on the resting potential of the large soma. Resting potentials are plotted against log. of external concentration. A; K. concentration. B; Ca concentration. The lines represent the average.

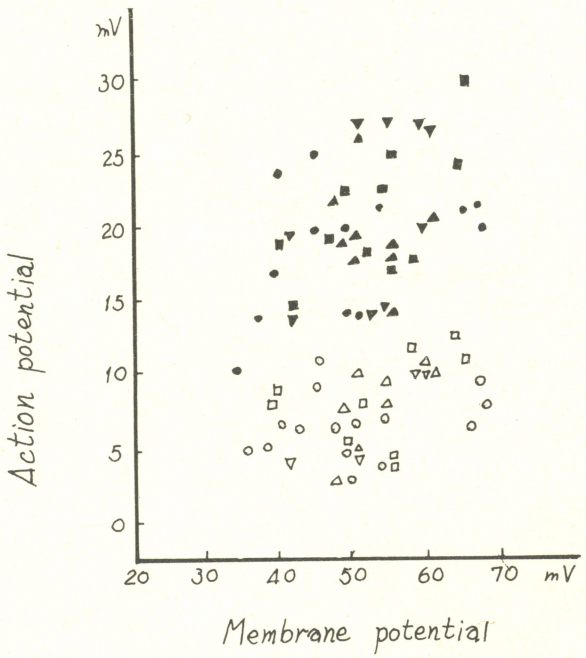

Fig. 5
Relation between resting & action potentials of the soma in the anterior four large cells.
● + ▲ ■ : maximal potentials of I, II, III, IV cells
○ x △ □ : sustained potentials of I, II, III, IV cells

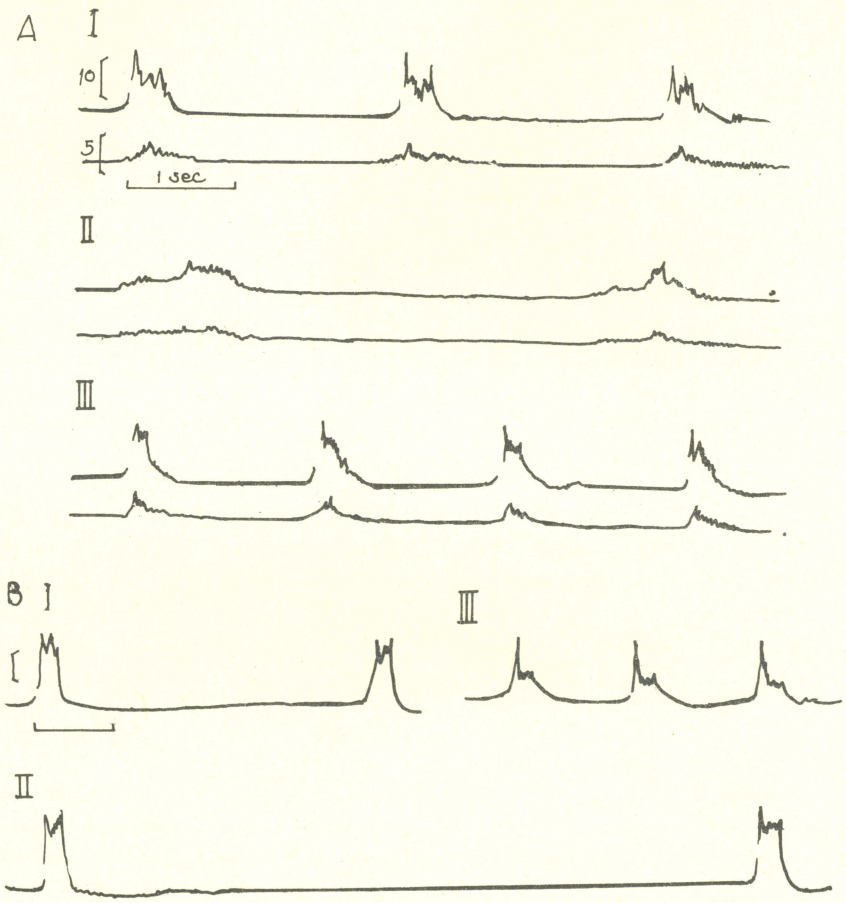

Fig. 6
Effects of excess Ca & excess K on the burst activity. A: Simultaneous recordings from I & V cell in a preparation of anterior four large cells. I: Normal, II, effect of 4 Ca III, effects of 4 K. Calibrations in B: 10 mv. 1sec.

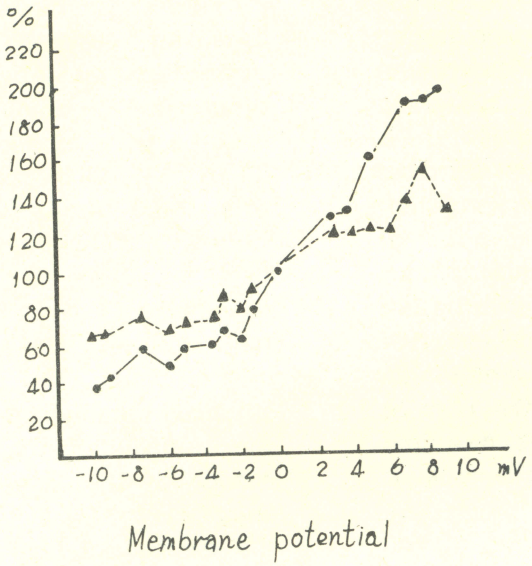

Fig. 7
Effects of K on the burst interval & amplitude of the sustained potential.
● : burst interval. ▲ : amplitude of sustained potential.

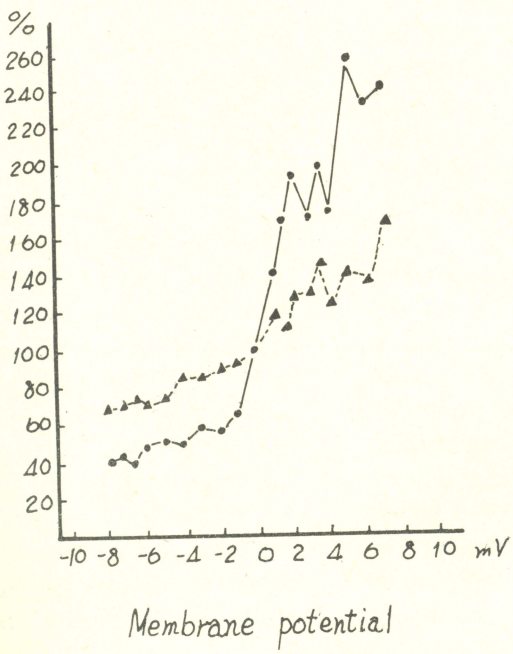

Fig. 8
Effect of Ca on the burst interval & amplitude of the sustained potential.
● : burst interval. ▲ : amplitude of sustained potential.

lobster (Hagiwara). [10]

The relation between the resting potential and external K concentration was shown in Fig. 4 A, in which the resting potentials were plotted against log. of external K concentration. It is shown that the resting potential decreases gradually as external K concentration increases. The slope of the potential change is small in low K concentrations and large in high K, which is probably related to the increased permeability of the membrane to K on account of depolarization. The slope of the part of higher K concentration was measured about 50 mv for a tenfold change in K concentration.

The relation between the resting potential of the soma and external Ca concentrations was shown in Fig. 4 B, in which the resting potentials were plotted against log. of Ca concentration. It is seen that the membrane potential increases gradually as external Ca concentration rises, the increment being less in concentrations higher than normal. The change in the membrane potential was about 10 mv for a fivefold decrease from in Ca concentration and about 5 mv for a fivefold increase in Ca concentration.

2. Effect of K concentrations on the activity of the soma:

The relation between the resting potential and the largest action potential as well as the sustained potential of the burst recorded in the soma was shown in Fig. 5. The largest action potential means the largest negative deflection occurred in the burst, which was usually consisted of synaptic and spike potentials. The data obtained from V cell (Matsui) [14] was discarded as the largest deflection followed by a sustained depolarization as seen in the anterior four large cells often failed to occure in that soma. The largest action potential in the soma was 30-15 mv, and the sustained potential about 4-15 mv. As seen Fig. 5. There were wide fluctuations in both potentials, according to preparations and to individual cells of the soma preparation. However, as a whole, it is shown in the figure that both largest and sustained potentials tend to increase as the resting potential increases. The change in the largest potential was about 2.5 mv for a change of 10 mv in the resting potential and that in the sustained potential was about 1.3 mv.

Excess of external K shortened the burst interval and duration and decreased the amplitude of the action potential along with the decrease in the resting potential (Fig. 6). It increased the frequency of spike and of synaptic potentials. In some preparations, it tended to initiate spontaneous spike during the inter burst, which was probably due to depolarization of the membrane of the cells, of which excitability were relatively low. Often in elevated the level of the after potential (Fig. 6 B) and often increased the slope of slow depolarization between the burst when it exsted (Fig. 6 B) Deficiency of external K generally affected the activity in a reversed way, though its effect on the burst component was often uncertain.

The relation between the burst activity and the resting potential of the soma when the preparation was affected by the change in external K concentration was

shown in Fig. 7. As the burst activity the burst interval and the magnitude of sustained potential in the burst were taken, and those were measured in the records showing regular activity.

In Fig. 7, relative changes in them were plotted respectively against increase or decrease in the resting potential. Fluctuations are seen in the figure especially in a region of higher resting potentials, which are probably due, in large part, to different susceptibility of the individual preparation. However, it is seen that both burst interval and magnitude of sustained potential becomes larger or smaller as the resting potential increases or decreases, and that the change in them becomes less in the region of higher or lower resting potentials. It is also seen that the burst interval is more affected than the magnitude of sustained potential.

3. Effects of Ca concentrations on the activity of the soma:

Excess of Ca lengthend the burst interval and duration along with the increase in resting potential (Fig. 6). It increased the amplitudes of large and small deflections and of sustained potential (Fig. 6 B). High Ca solution often depressed the burst and of the cell abruptly or gradually, while the resting potential continued to increase gradually to a steady value (Fig. 6 A). It often decreased the slope of slow depolarization between the burst when existed. Deficiency of Ca shortened the burst interval and duration of slow potential change and often produced a train of spontaneous spikes during the interburst. In some cases, an undulatory potential change took place when the medium was changed from high Ca solution to normal per fusion solution.

The relation between the burst activity and resting potential in the soma as affected by the change in Ca concentrations was obtained by the same way as in the case of K. This was shown in Fig. 8. Both burst interval and magnitude of sustained potential became larger or smaller as the resting potentials increased or decreased and the increment was relatively small in the region of higher or lower resting potential. The change in the magnitude of the sustained potential by low Ca was about the same with that by high K while the change by high Ca was larger than that by low K. on the other hand, the change in the burst interval by Ca solution was larger in comparison with that by K solution.

Discussion

The resting potential of the large soma, of which value accorded well with those obtained in various kinds of crustaceans (Hagiwara and Bullock), [3, 10, 15] is low compared with those obtained from axons of marine crustaceans. For instance, in Carcinus axon, it is about 82 mv, [12] in Homarus giant axon about 70 mv, [5] and in callinectes motor axon about 82 mv. [20]

The relation between the resting potential of the soma and external K concentration was similar to those obtained in crustacean axons, though the slope of the resting potential in the soma was somewhat less, which indicates that the resting potential of the soma is not accounted for well on the basis of the change in K potential alone. In excess Ca,

the resting potential of the soma increased as Ca concentration increased, though the slope of change between them was less compared with that in deficiency of K. This differs from the results obtained in a lobster nerve fibre, in which the saturation effect of Ca on the resting potential is shown in the external Ca concentration above 20 mv. [5]

These results obtained in the large soma may be explained by a general view, as discussed in detail by Shanes [16] that the resting potential of the soma is not regarded as a pure K concentration potential but is related to the permeability of other ions, which acts to different degree dependent on the membrane potential of the soma. Some complexity is brought about by the cable theory to interprete the relation between the resting potential and the activity of the large soma, because the resting potential of the soma must be compared with the deflections recorded in the soma which have been regarded as electrotonic spread from the loci outside the soma. [10] The loci of the spike, synaptic potential and sustained potential are thought to be various distances from the soma according to preparations and cells of the same preparation, and a large fluctuation in the potential changes as shown in Fig. 3 may be attributable to this. However, the amplitudes of deflections recorded in the soma, as a whole, are assumed to reflect correctly the potential of the axon which affects the electrotonic spread is thought to be correlated intimately to that of the soma. If this is correct, smaller slope of the sustained potential of the soma, plotted against the resting potential in comparison with that of the maximal potential (Fig. 3) will indicate that the loci of the sustained potential are, as a whole, more distant from the soma, or at their loci, the sustained potential is less affected by the resting potential than the maximal potential.

Change in the magnitude of sustained potential per unit change in resting potential was shown to be greater in high Ca solution than in low K solution. Similar result has been found in the action potential of the lobster giant axon, in which greater change in the action potential is produced by low Ca than by high K. [6] Above change in the soma may be related, in part, to the change in the electrotonic spread of the deflection due to the change in the membrane resistance by external ions, as the external K and Ca concentration affect the resistance of the crustacean axon membrane. [12] However, a possibility is pointed out that this is related to the effect of high Ca concentration on the inactivation of the Na permeability mechanism. It has been shown by weidmann [21] in purkinje fibre of the mammalian heart and by Frankenhaeuser and Hodgkin [8] in giant axon of Loligo, that the inactivation curve is shifted along the voltage axis by changing the external Ca concentration so that the fraction of the Na carring system is decreased in external Ca concentration.

The effect of external Ca or K concentration on the burst interval as generally the same with those of the provious result obtain by the investigation using extracellular recording. [13] It was shown that change in the interval of the burst per unit change in the resting potential of the soma was greater in external Ca than in external K. The interval of burst is

thought to be dependent on the resting potential of the cell, absolute critical potential for the burst formation, and the slow depolarization during the interburst when exists. In this experiment, when the slow depolarization was present, its slope was sharpened by high K and flattened by high Ca, so this factor is not concerned with the burst interval. The effect of high Ca or low K on the absolute critical level for the burst formation is not known in this experiment. However there are many evidences that this factor plays an important role. It is known in marine crustacean axon that the excess K causes a rise in the threshold, [19] and the lack of Ca results in decreased critical firing level and the excess of Ca depresses excitability. [20] In squid axon, the threshold is relatively insensitive to K except at extreme concentrations, [9] and in purkinje fibre of the mammalian heart, high Ca decreases excitability by lowering the threshold potential. [21]

Acknowledgments

The author wish to express his sincere thanks to Prof. K. Matsui, Head of Zoological Institute, Tokyo Kyoiku University (Japan), for his encouragement and guidance and providing the experimental facilities; to Prof. A. EBARA, for their valuable advice; to Dr. N. Ai. for his technical assistance and helpful advice.

This study was supported by a grant from National Science Council (Republic of China).

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若干離子濃度變化對龍蝦心臟神經節電位變動的影響

翁國榮

本研究係利用日本龍蝦（Panulirus japonicus）觀察細胞外鉀及鈣離子濃度的變化對其心臟內側神經節細胞電位變動的影響。

茲將其結果摘要如下：

(1)龍蝦心臟神經節的活細胞靜止電位介於33 mv至65mv之間，但多呈50至55mv。活細胞的 burst 振幅之最大電位及持續電位（sustain potential）可隨著細胞的靜止電位而增加。其增加程度卽前者比後者爲大。

(2)當細胞外鉀及鈣的離子濃度業已發生改變之數分鐘內，細胞的靜止電位與持續電位在此期間仍可保持穩定。

(3)過多的鉀可降低膜電位，並能縮短間隔及 burst 之持續而且可降低活動電位之振幅。高濃度的鉀在 interburst 中開始有自發性 spike，缺鉀對於 burst 的活性有相反效果。

(4)過多的鈣可增加膜電位，延長 burst 之間隔及持續，並且增加動作電流之振幅。高濃度的鈣常常可降低細胞活性，細胞外缺鈣時卽有相反效果。

(5)根據細胞外的鉀離子濃度及活細胞靜止電位的相互關係可證實活細胞的膜電位並不能作爲計量鉀電位之變化的基礎。

(6)從活細胞外鈣離子濃度與細胞靜止電位的關係言，鈣的安定作用力量顯然很小。

(7)就改變每一單位膜電位之 burst 間隔言，鈣溶液比鉀溶液的作用要強。

(8)高鈣溶液比低鉀溶液在活細胞的膜電位上，能有效地改變每一單位之持續電位（sustain potential）的振幅。

台北醫學院生物學科

民國67年5月19日受理