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Synaptic contribution to the production of the plateau formation of the biopotential of somatic neuromembranes treated with convulsants: experiment on the identified giant neurone of a snail's subesophageal ganglion-complex

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Synaptic contribution to the production of the plateau formation of the biopotential of somatic neuromembranes treated with convulsants: experiment on the identified giant neurone of a snail's subesophageal ganglion-complex*

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Abstract

I) On an identified giant neurone of the right parietal ganglion in a snail's subesophageal ganglion-complex, the synaptic contribution to the production of the plateau formation of biopotential or grouped spike discharges of the soma has been studied in the presence of a convulsant. 2) The orthodromic stimulation of a peripheral nerve (the intestinal nerve) can elicit the plateau formation of biopotential, instead of normal spike discharges, in the identified neurone treated with a convulsant. 3) With the application of a convulsant, for example bemegride which was in a concentration less than that necessary to produce the plateau formation, an EPSP accompanied a spike with a constant delay. This EPSP is a product of a proprioceptive reflex arc consisting of two excitatory synapses with a certain subordinate neurone. 4) Later, in the presence of a convulsant, spontaneously conveyed multiple EPSP's were observed on the biopotential of the identified neurone. These multiple EPSP's produced grouped spike discharges or the plateau formation of biopotential of the neurone. 5) The multiple EPSP's may be produced by the grouped spike discharges of the subordinate neurone, the membrane property of which would be changed by a convulsant. It is presumed that the grouped spike discharges or the plateau formation of biopotential often occurs synchron. ously in many neighboring neurones by means of synaptic triggering in the presence of a convulsant.

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SYNAPTIC CONTRIBUTION TO THE PRODUCTION OF THE PLATEAU FORMATION OF THE BIOPOTENTIAL OF SOMATIC NEUROMEMBRANES TREATED WITH CONVULSANTS: EXPERIMENT ON THE IDENTIFIED GIANT NEURONE OF A SNAIL'S SUBESOPHAGEAL GANGLION-COMPLEX

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Without a doubt, synaptic transmission greatly contributes to the fact that group discharges of spikes by many neurones occur simultaneously in the epileptogenic focus caused either by the application of convulsants to or by the injury of the mammalian central nervous system. Many physiologists concerned with mammalian brain have agreed (see Discussion in this paper) that the most important characteristic of the epileptogenic neuronal biopotential is the group discharges of spikes with the long-lasting excessive depolarization of the somatic neuromembrane. Some physiologists have attempted to clarify the epileptogenic mechanism at the neuronal level using the simple neuronal structures of lower animals. WASHIZU, *et al.* (12, 13), examined strychnine on the isolated stretch receptor neurone of the crayfish, and later CHALAZONITIS and TAKEUCHI (2) studied the action of metrazol on the identified giant neurone of the subesophageal ganglion-complex of the snail. They reported also that the group discharges of spikes with long-lasting excessive depolarization (the plateau formation of the biopotential) were observed in the presence of convulsants on invertebrate neurones in concordance with those observed on mammalian epileptogenic neurones. Two explanations are possible on the genetic mechanism of the abnormal excessive depolarization (see also Discussion): the property change of the neuromembrane, and the production of the huge excitatory synaptic activation caused by the treatment with convulsants. For several reasons, the author believes that the plateau formation of neuronal biopotential is produced by the property change of the treated neuromembrane, at least on the identified molluscan giant neurone. However, the plateau formations of the treated neuromembrane can be triggered by many depolarizing motives: a spontaneous depolarizing shift (produced by a so-called generator current) of the neuromembrane, synatic excitatory activation, or an artificial depolariz-

ing-current injection into the soma.

Attempting to follow the admirable work of Prof. HAYASHI (6) on the neuropharmacology of mammalian epilepsy through some observations of the epileptogenesis of molluscan giant neurones, the author will limit his interest in this paper to the way in which the synaptic activation contributes to the production of the plateau formation of the biopotential of molluscan giant neurone treated with convulsants.

MATERIALS AND METHODS

A neurone in the right parietal ganglion of the dissected subesophageal ganglion complex of the snail (Helix pomatia) was identified and used throughout the experiments mentioned in this paper. It is one of the largest neurone in this ganglion (nearly 200 μ in diameter), usually showing regular spontaneous spike discharges (almost once per second). The duraton of its action potential is the shortest among the neurones in this ganglion complex (nearly 3 milliseconds). The most important feature for the identification of this neurone was its anatomical characteristic, the fact that it sends four axonic branches into four peripheral nerves: two branches of the left pallial nerve, the anal nerve, and the right pallial nerve (Fig. 1). The identification of this neurone was made easily by means of the

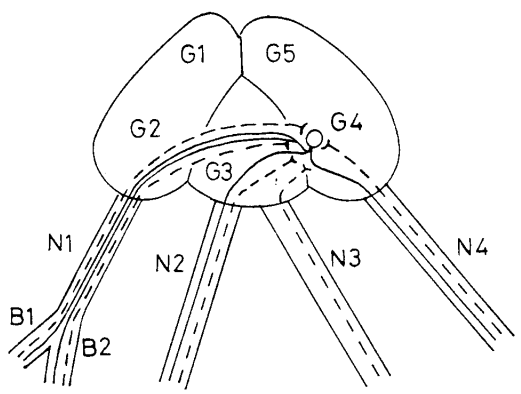

Fig. 1. A schematic diagram of the subesophageal ganglion-complex and identification of the neurone examined in this work

G1 : left pleural ganglion
G2 : left parietal ganglion
G3 : abdominal (visceral) ganglion
G4 : right parietal ganglion
G5 : right pleural ganglion
N1 : left pallial nerve
B1 : branch I
B2 : branch II
N2 : anal nerve
N3 : intestinal nerve
N4 : right pallial nerve

simultaneous recording of its somatic responses and its axonic impulses. Electrical stimulation of the intestinal nerve provokes the EPSP of the identified neurone.

Two micro glass pipettes filled with 2.5 M potassium chloride were implanted into the soma. One was used to record the intracellular neuronal potential using a oscilloscope, and the other was employed to change the neuromembrane polarization by a connection with an electronic stimulator (Fig. 2). To record

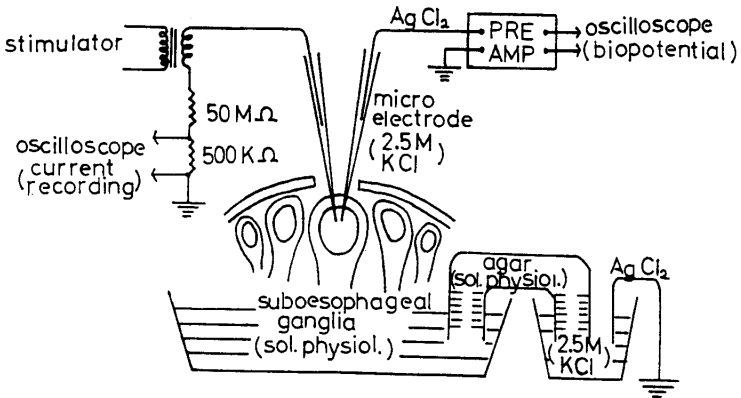

Fig. 2. A diagram of the experimental method

the axonic impulses in a peripheral nerve, a bipolar electrode of two fine silver wires (2–3 mm apart) was used. Convulsants were dissolved in a physiological solution for Helix pomatia, which was formulated by CARDOT (1). The pH values of these drug solutions were between 6.0 and 8.0. The temperature of the dissected ganglion was kept between 20° and 25°C during the experiment.

The identified neurone implanted with two micropipettes was usually in good condition for more than 4 to 5 hours, long enough to pursue the experiment.

RESULTS

In the presence of a convulsant in adequate concentration, the plateau formation of the identified neuromembrane is elicited by an electrical stimulation of the intestinal nerve of the subesophageal ganglion-complex, although this stimulation produces EPSP's with normal spikes from the same neurone under normal conditions (Fig. 3). It is impossible to consider that the plateau-formation of biopotential might consist of bombardments of EPSP's. The EPSP's which is orthodromically provoked by nerve stimulation only triggers the plateau formation of a neuromembrane treated with convulsants.

No convulsant examined by the author showed a depolarizing effect on the identified neurone. But some of them excited the other neurones

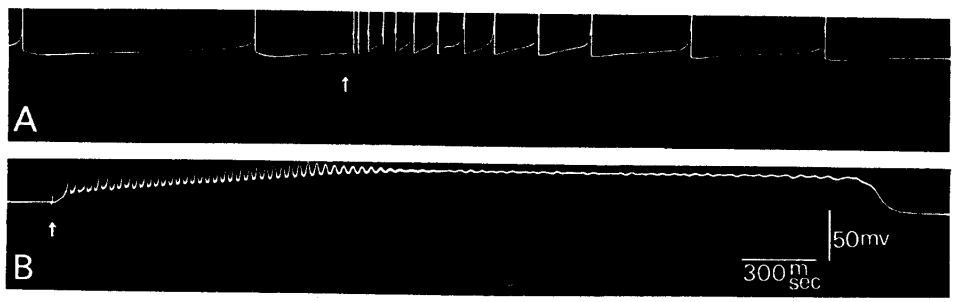

Fig. 3. The effects of orthodromic nerve stimulation (intestinal nerve)
A : Under normal conditions
B : 30 min. after the application of 90 mM metrazol

in the dissected ganglion, and consequently the synaptic influences were conveyed to the identified neurone.

Bemegride, strychnine and sometimes metrazol in a concentration less than that necessary to produce the plateau formation of biopotential caused an accompanying EPSP which followed the spontaneously firing spike with a constant delay (Fig. 4). The scheme of neuronal connections

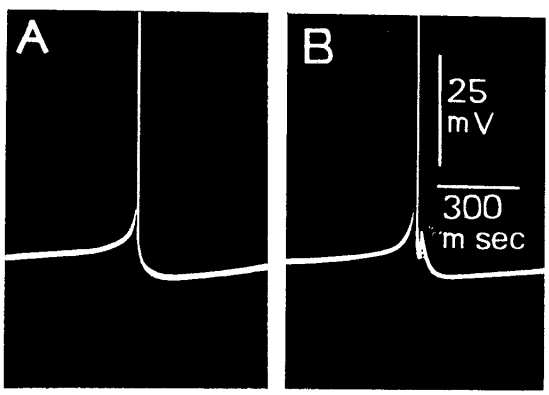

Fig. 4. An accompanying EPSP just after a spike in the presence of bemegride
A : Under normal conditions
B : 7 min after the application of 45 mM bemegride

shown in Fig. 5 must be accepted to explain such an accompanying EPSP just after the spike. As shown in this scheme, the identified neurone (the recorded neurone, (C1) joined a subordinate neurone (C2) with an excitatory synaptic connection (S1), and the subordinate neurone also connected the identified neurone with an excitatory synapse (S2), making a proprioceptive reflex arc from the identified neurone back to itself via a subordi-

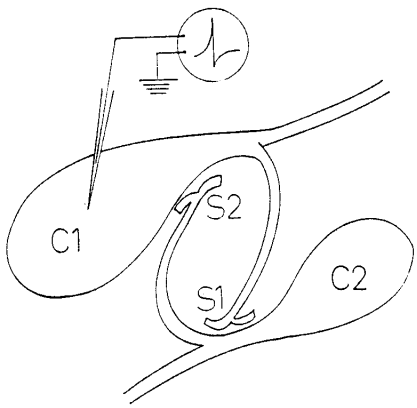

Fig. 5. A diagram of neuronal connections to explain the accompanying EPSP occurrence (see text)
C1 : identified neurone, biopotential of which was intracellularly recorded
C2 : subordinate neurone
S1 : excitatory synaptic connection
S2 : excitatory synaptic connection

nate neurone. Under normal conditions, the subordinate neurone is silent, and its EPSP provoked by the activation of S1 is insufficient to produce the spike. However, bemegride, for example, facilitated the activation of S1, and the subordinate neurone produced a spike discharge which brought an EPSP to the identified neurone by means of the activation of S2. Furthermore, in the presence of bemegride, multiple EPSP's were able to be conveyed and were able to provoke grouped spike discharges by the identified neurone (Fig. 6). The frequencies of the conveyed

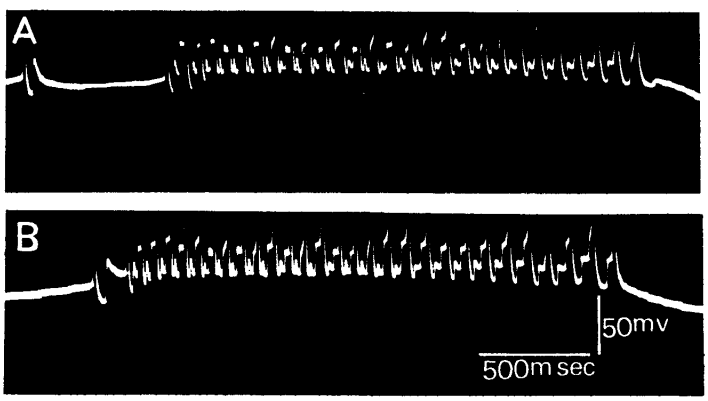

Fig. 6. Grouped spike discharges produced by conveyed multiple EPSP's
A : 10 min after the application of 45 mM bemegride
B : 11 min after

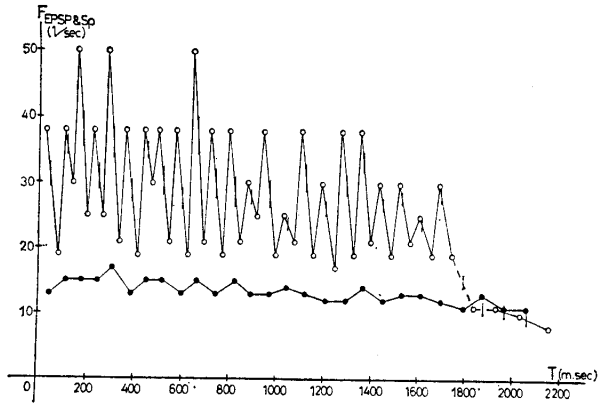

Fig. 7. Frequencies of conveyed multiple EPSP's and produced spike discharges shown in Fig. 6, A. (The plateau formation could not be produced in this case.)

Ordinate : frequencies of conveyed EPSP's (per second) [open circle (○)] (Vertical short line means the occurrence of a spike.) ; frequencies of produced spikes (per second) [closed circle (●)].

Abscissa : time course (in m. sec.).

multiple EPSP's and spike discharges by the identified neurone in these cases are shown in Fig. 7 and Fig. 8. In these cases, two EPSP's were found in each interval of two spikes. And EPSP frequencies, as a general rule, decreased gradually toward the end of multiple EPSP discharges.

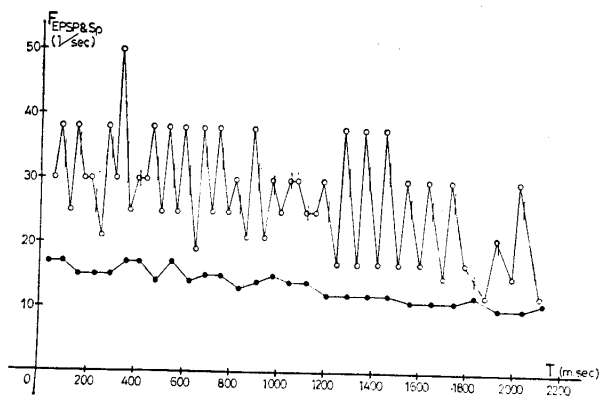

Fig. 8. Frequencies of conveyed multiple EPSP's and produced spike discharges shown in Fig. 6, B. (The plateau formation could not be produced in this case.)

Ordinate : frequencies of conveyed EPSP's (per second) [open circle (○)] (Vertical short line means the occurrence of a spike.) ; frequencies of produced spikes (per second) [closed circle (●)].

Abscissa : time course (in m. sec.).

Later, with the presence of bemegride, grouped spike discharges which were evoked by multiple EPSP's changed to the plateau formation of biopotential (Fig. 9). Frequencies of the conveyed EPSP's and the evoked spike discharges were calculated graphically as shown in Fig. 10 and Fig. 11. At the beginning of the multiple EPSP bombardment, three EPSP's could be observed in each interval of two spikes, and in the later

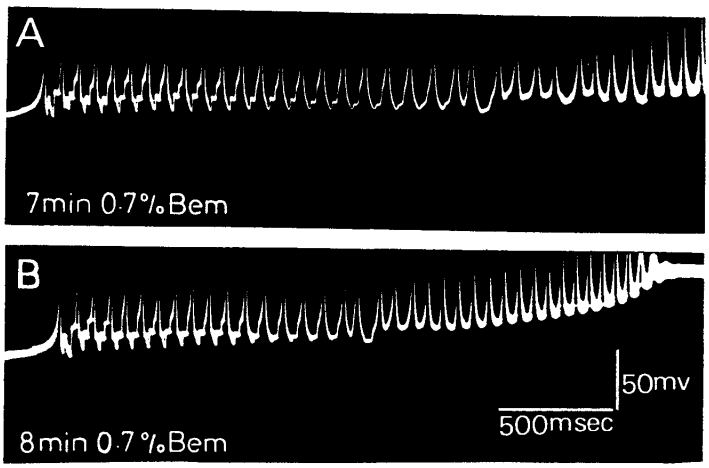

Fig. 9. Grouped spike discharges produced by conveyed multiple EPSP's, which continued up to plateau formation
A : 7 min. after the application of 45 mM bemegride
B : 8 min. after

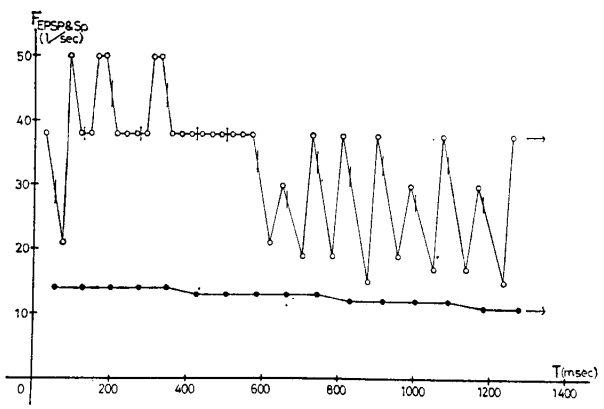

Fig. 10. Frequencies of conveyed multiple EPSP's and produced spike discharges shown in Fig. 9, A. (These spikes continued up to plateau formation.)
Ordinate : frequencies of conveyed EPSP's (per second) [open circle (○)] (vertical short line means the occurrence of a spike.) ; frequencies of produced spikes (per second) [closed circle (●)].
Abscissa : time course (in m. sec.).

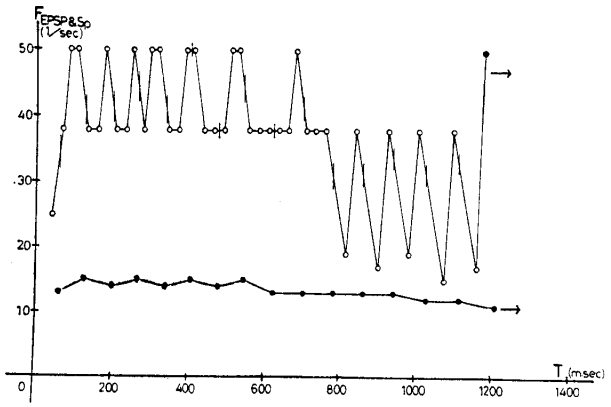

Fig. 11. Frequencies of conveyed multiple EPSP's and produced spike discharges shown in Fig. 9, B. (These spikes continued up to plateau formation.)
Ordinate : frequencees of conveyed EPSP's (per second) [open circle (○)] (vertical short line means the occurrence of a spike.) ; frequencies of produced spikes (per second) [closed circle (●)].
Abscissa : time course (in m. sec.).

part of grouped spike discharges, two EPSP's were found between two spikes. When three EPSP's were found between two spikes, the frequencies of conveyed EPSP's were almost constant. If we suppose that in the later part of such grouped spike dischatges a third EPSP which follows two visible EPSP's would be hidden behind a evoked spike, we could presume the frequencies of all the conveyed EPSP's to be constant. If the frequencies of EPSP conveyed to the identified neurone are almost constant under such a postulation, it can be presumed consequently that the subordinate neurone will show grouped spike discharges produced by the plateau formation of the biopotential, implying the property change of its neuromembrane caused by bemegride.

Grouped spike discharges of the identified neurone shown in photographs of Fig. 6 were apparently provoked by the bombardment of multiple EPSP's, not produced by the membrane property change of the identified neurone. However, in cases shown in Fig. 9, the spike discharges which were provoked by multiple EPSP's from the subordinate neurone triggered the plateau formation of biopotential in the identified neurone due to a membrane property change in the identified neurone. When a remarkable depolarization of the neuromembrane of the identified neurone occurred just before the completion of plateau formation, conveyed EPSP's apparently disappeared completely, being hidden by such a biopotential shift.

It is presumable that the grouped spike discharges or the palteau

formation of biopotential may often occur in the mammalion brain simultaneously in many neighboring neurones by means of synaptic triggering, in the presence of some convulsant or in the chronic epileptogenic focus, as has been described in this paper in cases relating to the molluscan dissected ganglion.

A question remained on the relationship between the somatic response and the axonic impulse during the occurrence of grouped somatic spike discharges in the presence of a convulsant, so a supplemental figure has been added (Fig. 12). According to this experiment, axonic impulses appeared synchronously to somatic spikes firing in a group with the application of a convulsant (Fig. 12, B). After the completion of plateau formation of somatic biopotential, axonic impulses were observed synchronously to the somatic local responses (Fig. 12, C).

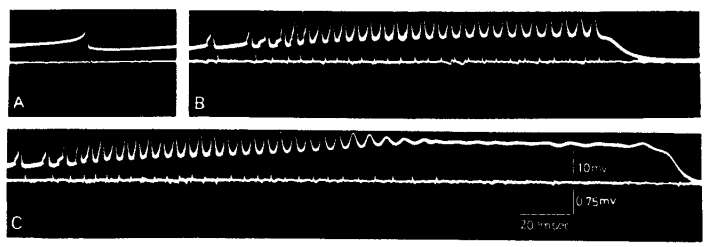

Fig. 12. Simultaneous recording of somatic response and axonic impulse
Upper beam : somatic response.
Lower beam : axonic impulse (left pallial nerve)
A : Under normal conditions
B : 29 min after the application of 90 mM metrazol
C : 30 min after

DISCUSSION

The discussion in this paper will be concerned with the comparison between the biopotential of a molluscan giant neurone treated with a convulsant and that of a mammalian epileptogenic neurone in epileptic focus caused by several methods.

Concerning the mammalian cortical neurone, ENOMOTO and AJMONE-MARSAN (5) reported that the grouping burst of highly frequent spikes occurred in epileptogenic focus, in paralled with the paroxysmal EEG discharge. MATSUMOTO and AJMONE-MARSAN (7) (8) recorded intracellularly the neuronal biopotential in the epileptogenic focus of a cat's cortex, and observed that the transient depolarization of neuromembrane, "paroxysmal depolarization shift (PDS)" which was followed by a long lasting

hyperpolarization would be important characteristics of the epileptogenic biopotential in the interictal period. The shape of PDS in a mammalian cortex resembles that of the plateau formation of the molluscan giant neurone described above, in spite of two differences, the duration of the transient depolarization and the size of after hyperpolarization.

MATSUMOTO, AYALA and GUMNIT (9) studied the epileptogenic phenomenon upon the pyramidal tract neurone, and reported that the somatic membrane depolarization caused by passing transmembrane current could never elicit PDS. Only orthodromic stimulation (stimulation of thalamic VL-nucleus) but not antidromic stimulation could provoke PDS in all-or-none manner. The membrane depolarization produced by the current injection into the soma could cause only an amplitude diminution of PDS, which was provoked only by stimulation of thalamic VL-nucleus. Conversely, the membrane hyperpolarization could bring about an amplitude augmentation.

On the other hand, CHALAZONITIS and TAKEUCHI (2) have already reported that the depolarizing current injection into the soma of the molluscan giant neurone could decrease the intervals of plateau formations which repeated regularly. Conversely also, the hyperpolarizing current injection could prolong the intervals of plateau formations on such a molluscan neurone.

MATSUMOTO, *et al.* insisted that PDS of the mammalian neurone would be a synaptic event, a huge excitatory synaptic potential provoked only by orthodromic stimulation. However, a question remained whether the current injected into the soma of mammalian neurone was capable of extending to the arborized dendrites where PDS would originate. On the contrary, the soma of a molluscan giant neurone is simply oval without any dendrites, and the injected current spreads easily into the entire soma up to the axon hillock portion where the plateau formation would be initiated. In spite of some difference of electrophysiological features between the PDS of the mammalian epileptogenic neurone and the plateau formation of the molluscan neurone treated with convulsants, the two electrophysiological phenomena resemble each other greatly.

Further, concerning the mammalian epileptogenic neurone, PRINCE (10, 11) studied it in the cerebral cortex, and DICHTER and SPENCER (3, 4) examined it in the hippocampus, announcing together a similar result to that obtained by MATSUMOTO, *et al.*

On the side of the invertebrate neurone, the genetic mechanism of plateau formation caused by a convulsant must be explained by the property change of the neuromembrane treated by the drug and not by

the abnormal synaptic event, because plateau formations occurred very regularly in repetition and were controled by the membrane polarization change produced by a current injection. However, synaptic activation could trigger plateau formation, and many neurones treated with a convulsant could display simultaneously the grouped spike discharges or the plateau formation by mutual synaptic triggering.

SUMMARY

1) On an identified giant neurone of the right parietal ganglion in a snail's subesophageal ganglion-complex, the synaptic contribution to the production of the plateau formation of biopotential or grouped spike discharges of the soma has been studied in the presence of a convulsant.

2) The orthodromic stimulation of a peripheral nerve (the intestinal nerve) can elicit the plateau formation of biopotential, instead of normal spike discharges, in the identified neurone treated with a convulsant.

3) With the application of a convulsant, for example bemegride which was in a concentration less than that necessary to produce the plateau formation, an EPSP accompanied a spike with a constant delay. This EPSP is a product of a proprioceptive reflex arc consisting of two excitatory synapses with a certain subordinate neurone.

4) Later, in the presence of a convulsant, spontaneously conveyed multiple EPSP's were observed on the biopotential of the identified neurone. These multiple EPSP's produced grouped spike discharges or the plateau formation of biopotential of the neurone.

5) The multiple EPSP's may be produced by the grouped spike discharges of the subordinate neurone, the membrane property of which would be changed by a convulsant. It is presumed that the grouped spike discharges or the plateau formation of biopotential often occurs synchronously in many neighboring neurones by means of synaptic triggering in the presence of a convulsant.

RÉSUMÉ

1) Sur un neurone géant identifié du ganglion pariétal droit dans le ganglion-complexe sous-oesophagien d'escargot, la contribution synaptique pour produire la formation de plateau du biopotentiel somatique ou décharges groupées des pointes somatiques a été etudié dans la présence de quelques convulsivants.

2) La stimulation orthodromique du nerf périphérique (le nerf in-

testinal) a pu évoquer la formation de plateau du biopotentiel du neurone traité par le convulsivant, au lieu des décharges des pointes dans les conditions normales.

3) Avec l'application des quelques convulsivants, bémégride par exemple dans la concentration sous-liminale pour produire la formation de plateau, un EPSP était accompanié par une pointe avec un délai très constant. Ce EPSP doit être un produit du arc de réflex propriocéptif consistant aux deux synapses excitatrices via un certain neurone subordonné.

4) Plus tard dans la presence du convulsivant, les multiples EPSPs spontanément arrivés ont pu être observés sur le biopotentiel du neurone identifié. Ces multiples EPSPs ont pu produire les décharges groupées des pointes ou la formation de plateau du biopotentiel du neurone.

5) Les multiples EPSPs doivent être dûs aux décharges groupées des pointes du neurone subordonné, la nature neuromembranaire duquel serait changée par le convulsivant. Il est à présumer que les décharges groupées des pointes ou la formation de plateau du biopotentiel doivent se produire synchronement dans plusieurs neurones du même foyer au moyen de "triggering ' synaptique dans la présence du convulsivant.

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