

contraction was induced by  $5 \times 10^{-6}$  M acetylcholine-chloride solution.

In the case when tonic muscle fibres are kept for 1 h in R + 200G, their contraction capacity after glycerol removal is found to be reduced about twice, and 2 h later is restored completely. If after that the tonic fibres are transferred to R + 400G for 1 h and washed out once more, the contraction retains. As was demonstrated on 5 fibres under investigation the amplitude of contraction in 1 h after the second glycerol removal was 20–40% of the original value (Table). Thus the preliminary removal of glycerol lower concentration reduced the disconnection effect of higher concentration. The same effect was obtained in 6 experiments on phasic fibres.

The ultrastructure of the isolated tonic fibre undergoes considerable changes after 1-hour incubation in R + 400G followed by 2-hour wash out in the Ringer solution. Large vacuoles can be seen between myofibrils in sites of localization of sarcoplasmic reticulum elements. Moreover, small vacuoles occur inside myofibrils. The membranes around these vacuoles could not be seen (Figure 2). In the second experimental variant, when the fibre was kept in R + 200G and then re-incubated in R + 400G, alterations in the ultrastructure were not so strong. There were no small vacuoles inside myofibrils. Large vacuoles were rare and, as a rule, retained their membranes.

To understand the difference between our data and the results obtained by STEFANI and STEINBACH<sup>13</sup> we per-

formed experiments on muscle bundles consisting of about 15–20 various fibres. In this case, as in the experiments of the above authors, the phasic contraction component disappeared completely after the removal of 400 mM glycerol, while tonic contraction was retained. It appears that different effects of glycerol removal on isolated fibre and bundle of fibres are caused by variation in the rates of glycerol removal. There is evidence that the low speed of glycerol efflux does not destroy the T-system<sup>12</sup>. Thus it was shown that tonic fibres are more resistant to the deleterious effect of glycerol removal than phasic ones (Table).

As in the case of phasic fibres, the tonic fibre membrane responds to high potassium ion concentration by depolarization without contraction of fibres. Caffeine, nevertheless, induces contraction. This indicates that in both types of the fibres ECC is dissociated. In this context it can be suggested that in tonic fibres, as in phasic ones, vacuoles are formed due to the T-system swelling. Thus it implies the same mechanism of glycerol removal effect on tonic and phasic fibres. Since there is no essential difference in the diameter of tonic and phasic fibres, it is hard to suppose that in the former case activation of contraction is accomplished directly by the surface membrane. The slowness of contraction may be explained by scarce T-system elements as compared with phasic muscle fibres<sup>7,8</sup>. Moreover, the appearance of vacuoles during glycerol removal need not have prevented excitation spreading inside the fibre had it been brought about by direct diffusion of activator from the surface membrane.

Thus the above evidence shows that there is no essential difference between phasic and tonic fibres as regards the mode of spreading excitation from the membrane to contractile apparatus. Quantative difference in the development of the T-system appears to be responsible for higher resistance of tonic fibres to glycerol removal.

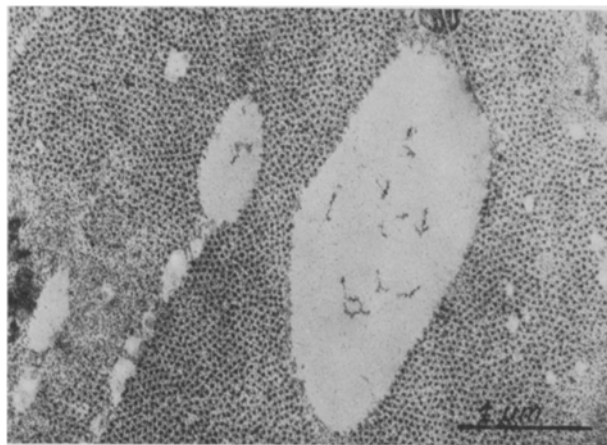


Fig. 2. Changes in the ultrastructure of frog tonic muscle fibres after incubation in glycerol solution (R + 400 mM G) for 1 h and its removal for 2 h. Fixation in 2.5% glutar aldehyde in cacodilate buffer at pH 7.2; Epon 812 embedding. GEM — 7;  $\times 25,000$ .

**ВЫВОДЫ.** На основании регистрации сокращений, мембранного потенциала и изучения ультраструктуры изолированных тонических мышечных волокон лягушки при действии глицерина делается заключение о том, что в этих волокнах, также как в фазных, T-система необходима для осуществления электромеханической связи.

G. A. NASLEDOV, J. E. MANDELSTAM and  
T. L. RADZJUKIEWICH

*Sechenov Institute of Evolutionary Physiology and Biochemistry, USSR Academy of Sciences, Thorez pr. 52, Leningrad K-223 (USSR, 194223), 4 April 1972.*

## D- and L-Isomers of Serine and Alanine Equally Effective as Releasers of Gastrin

We<sup>1</sup> recently reported that L-phenylalanine releases cholecystokinin but D-phenylalanine does not when solutions of them are perfused into the intestine. We report here that D- and L-isomers of serine and alanine are equally effective as releasers of gastrin when solutions of them bathe the mucosa of the pyloric gland area of the stomach. The studies were done on 3 dogs with vagally innervated pouches of the pyloric gland area (antrum) and vagally innervated pouches of the oxyntic gland area (Pavlov type). Solutions to be tested were introduced into the antral pouch and the effect on rate of secretion of

acid from the oxyntic gland pouch was measured. The amino acids tested, glycine, alanine, and serine, are known to be among the most effective ones in releasing gastrin<sup>2</sup>, and in the present tests the responses to these 3 amino acids did not differ significantly (Table). We found no previous reports comparing D- and L-isomers of amino

<sup>1</sup> J. H. MEYER and M. I. GROSSMAN, *Gastroenterology* 58, 1046 (1970).

<sup>2</sup> C. E. ELWIN and B. UYNAS, in *Gastrin* (Ed. M. I. GROSSMAN, Butterworths, London 1966), p. 75.

Peak rate of  $H^+$  secretion from oxyntic gland pouch in response to irrigation of antral pouch with solutions of amino acids

Solution in antral pouch	No. of tests	Peak $H^+$ response ( $\mu$ equiv/min)	
		Mean	Standard error
none	27	3.6	0.3
0.15 M NaCl	21	3.9	0.2
0.4 M L-serine	6	19.6	1.6
0.4 M D-serine	6	17.0	1.4
1.0 M L-alanine	6	18.5	1.7
1.0 M D-alanine	6	22.2	1.9
0.4 M glycine	6	19.1	1.6

Both pouches drained to the exterior through cannulas of the type described by GREGORY<sup>3</sup>. After an 18 h fast juice was collected continuously from the oxyntic gland pouch and divided into 15 min samples which were titrated with 0.2 M NaOH to pH 7.0 by glass electrode. Volume of test solution introduced into the antral pouch was in all instances 8 ml, found in preliminary studies to be the largest volume that did not release gastrin by distention. Contractions of the pouch moved the solution back and forth between the pouch and a reservoir connected to the cannula by a rubber tube<sup>4</sup>. The pH of the solutions introduced into the antral pouches was in all instances 7.0 and the pH of the solutions recovered was always greater than 6.0. The concentrations of amino acids used are those found in preliminary studies to give the highest response, that is, doubling the concentration gave no higher response. Test solutions were left in the pouch for 90 min. Peak response is taken as the highest rate of  $H^+$  secretion during any 1 of the 6 15-min collection periods. Peaks occurred at 45 to 90 min. An equal number of tests were done in each of 3 dogs. The order of testing the various substances was randomized. As an index of the secretory capacity of these pouches, the mean maximal response to 8  $\mu$ g/kg/h of penta-gastrin was 134  $\mu$ equiv/min.

acids as gastrin releasers. In the present study, for both serine and alanine the response to the D-isomer was not significantly different from that of the L-isomer (Table). Most biological systems distinguish between D- and L-isomers of amino acids. On of the few exceptions is the so-called sarcosine carrier<sup>5</sup> involved in intestinal transport of neutral amino acids; it shows equal affinity for D- and L-isomers. It is of great interest that of the amino acids tested in both systems those amino acids that show high affinity for the sarcosine carrier<sup>6</sup> are also effective releasers of gastrin<sup>2</sup>; this includes the 3 amino acids used in the present study<sup>7</sup>.

*Zusammenfassung.* D- und L-Isomere von Alanin und Serin sind beide wirksam, von der Antrumschleimhaut aus den Gastrinmechanismus zu aktivieren.

A. CSENDES and M. I. GROSSMAN

Veterans Administration Center, Building 115, Room 115, Los Angeles (California 90073, USA), 26 February 1972.

<sup>3</sup> R. A. GREGORY, J. Physiol., Lond. 111, 119 (1950).

<sup>4</sup> P. A. BURSTALL and B. SCHOFIELD, J. Physiol., Lond. 120, 383 (1953).

<sup>5</sup> V. G. DANIELS, H. NEWBY and D. H. SMYTH, Biochim. biophys. Acta 183, 637 (1969).

<sup>6</sup> V. G. DANIELS, H. NEWBY and D. H. SMYTH, Biochim. biophys. Acta 173, 575 (1969).

<sup>7</sup> This work was supported by Veterans Administration research funds and by grants from the US Public Health Service.

## Evoked Activity in the Nervous System of *Callinectes sapidus* Following Phasic Excitation of the Statocysts

The role of the crustacean statocyst in determining body orientation with respect to gravity has been clearly established (KREIDL<sup>1</sup>, SCHÖNE<sup>2,3</sup>, and DIJKGRAAF<sup>4,5</sup>). The work of COHEN<sup>6-8</sup> suggests that the tonic output of certain primary afferent fibers coming from statolith hair receptors inside the statocyst is responsible for the attitudes assumed by the appendages as an animal is rotated about one of its axes.

Of what importance then are the thread hair receptors found in decapod statocysts? Do they also play a role in orientation? It is known that they do not come in contact with the statolith and are, therefore, not directly affected by gravity.

The present study was undertaken in an attempt to clarify some aspects of the functional role of thread hair receptors in decapods. Toward this end first-order and high-order units sensitive to phasic excitation of the statocysts were investigated in the swimming crab, *Callinectes sapidus*. Brief postural changes which accompany phasic excitation of the statocysts are described.

*Materials and methods.* Statocysts were exposed by cutting away portions of the surrounding exoskeleton. A probe attached to a micromanipulator was used to rotate either statocyst through a fixed 3° arc in the vertical plane. Basal segments containing the statocysts are hinged in *Callinectes* permitting vertical rotation. Impulses were recorded from nerves leaving the statocysts and cerebral ganglion with the aid of a preamplifier and displayed on one channel of an oscilloscope. The duration

of the rotary stimulus was monitored on a second channel. Phasic evoked activity was photographed as a standing spot using a Kymograph camera.

*Results. Evoked activity in first-order units from the statocysts.* In *Callinectes* the central branches of the antennular nerve innervate the statocysts. Spontaneous activity in these branches is restricted to afferent impulses from receptors inside the statocysts. Phasic rotation of a statocyst typically evoked responses like those shown in Figure 1A and B. Many afferent units were found which responded with a brief burst of repetitive activity to phasic excitation of a single statocyst. Figure 1B indicates that first-order units responding to rotation of a statocyst in one direction do not respond to rotation in the opposite direction. Destruction of bipolar sensory neurons projecting to thread hairs inside the statocyst abolished phasic evoked activity.

*Evoked activity in high-order units originating in the central nervous system.* High-order units which respond to

<sup>1</sup> A. KREIDL, Akad. Wiss. Wein. Math-Nature K., Abt. 111, 102, 149 (1893).

<sup>2</sup> H. SCHÖNE, Verh. dt. zool. Physiol. Ges. Suppl. 16, 157 (1951).

<sup>3</sup> H. SCHÖNE, Z. vergl. Physiol. 36, 241 (1954).

<sup>4</sup> S. DIJKGRAAF, Experientia 12, 394 (1956).

<sup>5</sup> S. DIJKGRAAF, Z. vergl. Physiol. 38, 491 (1956).

<sup>6</sup> M. J. COHEN, J. Physiol., Lond. 130, 9 (1955).

<sup>7</sup> M. J. COHEN, Biol. Bull. 171, 318 (1956).

<sup>8</sup> M. J. COHEN, Proc. R. Soc. B. 152, 30 (1960).