CALCIUM AND THE EFFECTS OF DRUGS ON SMOOTH MUSCLE OF THE OESOPHAGUS OF *APLYSIA BRASILIANA* (MOLLUSC-OPISTOBRANCH) *

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11 Figs.

SUMMARY

The smooth muscle fibers of the oesophagus of *Apiysia brasiliana* are very sensitive to ACh. When stimulated by at least 1μg/ml of ACh, the muscle contracts rapidly and presents sustained contraction. Relaxation immediately follows washing. During sustained contraction the spontaneous movements are not abolished. ACh is blocked by Atropine. Under Nicotine the muscle presents very strong contraction and the relaxation time is very long.

Serotonine (5-hydroxytryptamine) also provokes contraction at weak concentration. The effects of ACh

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and that of Serotonine depend on the presence of $Ca^{2}+$ in the perfusion fluid.

The anterior third of the organ is not or is very little sensitive to ACh, but the posterior 2/3 present the same behaviour as the total organ. This fact is discussed.

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CÁLCIO E EFEITOS DE DROGAS SÔBRE A MUSCULATURA LISA DO ESÔFAGO DE *APLYSIA BRASILIANA* (MOLUSCO-OPISTOBRÂNQUIO)

SUMÁRIO

A musculatura do esôfago de *Aplysia brasiliana* estimulada pelo menos com 1 µg/ml de ACh contrae-se ràpidamente e apresenta contração sustentada. O relaxamento segue-se imediatamente após a lavagem da preparação. Durante a contração sustentada os movimentos espontâneos não se abolem. ACh é bloqueada pela atropina. A Serotonina provoca contrações em doses fracas. A Nicotina determina forte contração sendo longo o tempo de relaxamento. Demonstrou-se também que o efeito da ACh depende da presença de ions Cálcio no líquido perfusor, o mesmo ocorrendo com a Serotonina. Verificou-se ainda que o 1/3 anterior do órgão é pràticamente insensível à ACh e os 2/3 posteriores se comportam em relação a êste éster como órgão total. Êste fato é discutido.

1.

INTRODUCTION

The mechanism of contraction of the smooth muscle of Mammals is rather well known, but that of Invertebrates, chiefly the marine ones, is far behind that of Vertebrates. Visceral muscles of Mammals are preferred for physiological and pharmacological essays, and a large amount of data have been collected.

Several Invertebrates as for example, Echinoderms have only smooth muscles in the whole body, but some of them behave as the striated muscles of Vertebrates when stimulated by some drugs as e.g. Acetylcholine, Serotonine and others (Sawaya 1962).

Other Invertebrates; as the Molluscs carry on both types of muscles, smooth and striated (Hoyle 1957, p. 12; Hanson and Lowy 1960, p. 274).

The present paper is concerned with effects of calcium and some drugs on the oesophagus of *Aplysia brasiliana,* in order to contribute to understanding the complex mechanism of contraction of smooth muscles.

2.

MATERIAL AND METHODS

Aplysias were caught at the mangrove beach of Araçá near the harbor of São Sebastião (23°, 48' 77" S; 45°, 24' W) and immediately carried to the laboratory of the Institute do Marine Biology of the University of São Paulo, at the Segredo beach, 4 km South of São Sebastião. Fifty *Aplysia brasiliana* of 180 to 250 g in weight were opened by the ventral side and the oesopaagus of 5-8 cm in length exposed, isolated, cut and transferred to a perfusion bath.

Filtered sea water of pH 7,5-7,8, was used as perfusion fluid bubbled with oxygen.

As excitant ACh and other drugs as Atropine sulphate (Merck), Nicotine (Eastman Kodak), and Calcium chloride (Merck) were emploied.

For studying the effect of Ca^{2+} ion on the muscle fiber artificial sea water, according to Pantin (1948, p. 63) was prepared as follows: NaCl — 23, 427 g; KCl — 0.729 g; $CaCl_2$ $6H_2O$ — 2.218 g; $MgCl_3$ $6H_2O$ — 10.702 g; Na_2SO_4. H_2O 8.967 g; $NaHCO_3$ — 0.210 g; NaB. $2H_2O$ — 0.079 g.

To balance the ionic content of the perfusion fluid, according to ionic amount of sea water of the same place where the Aplysias were caught, the following artificial sea-water was used: NaCl 21,48 g; KCl 0.745 g; $CaCl_2$. $2H_2O$ — 1,472 g; $MgCl_2$ 6,652 g; Na_2SO_4 5,03 g; NaBr 0,634 g; $NaHCO_3$ 1,278 g.

After some preliminary experiments, attention has been called to the different behaviour of two halves of the oesophagus, and to clarify this point the organs were cut into two parts, each one used separately in the perfusion bath. For recording isometric and isotonic contractions a lever of Schild type has been ajusted to a kymograph. All experiments were done at room temperature between 20 and 24° C.

3.

EXPERIMENTS

1. *Spontaneous contractions*:

Left in the perfusion bath the oesophagus usually shows a series of rythmic spontaneous contractions (Fig. 1). The amplitude of them varies very much but after some hours it is possible to see some periodism.

Fig. 1

2. *Effects of drugs*:

a) *Acetylcholine* — stimulated by ACh the oesophagus contracts immediately and sustains the contractions in which the spontaneous contractions are still evident, but smaller than the normal ones. The threshold of contraction under ACh is 1 μg/ml (Fig. 3-6). The muscle relaxes immediately after washing, but the spontaneous contractions decrease. The tension of contraction is about 2 g (Fig. 3). The amplitude of the responses to ACh depends on the concentration of the ester (Fig. 1-2-4). Fig. 4 shows also this relation.

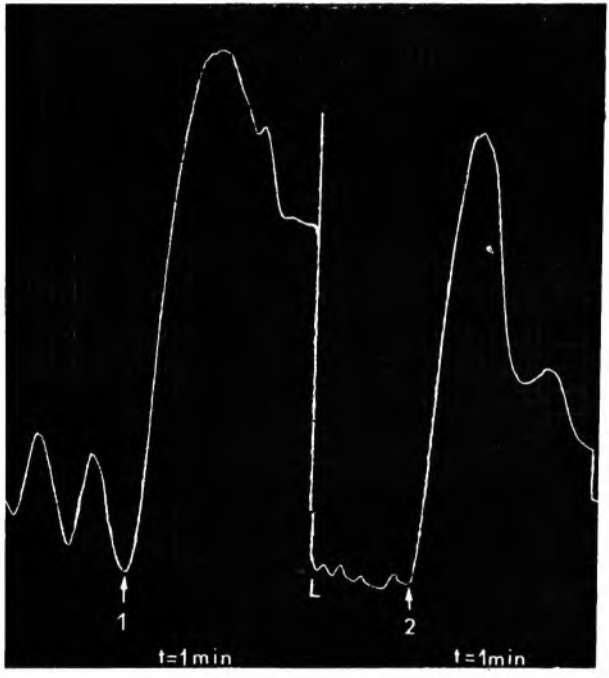
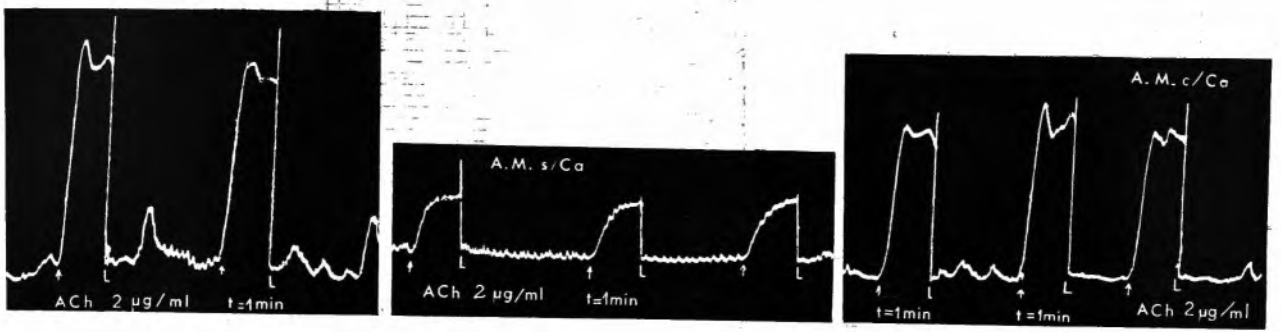


Fig. 1 — Spontaneous movements of the oesophagus of *Aplysia brasiliana* and stimulation by ACh: 1 = 2 μg/ml; L = washing; 3 = 2 μg/ml.



A B C

Fig. 2 — Contraction of the oesophagus of *A. brasiliana*.
A — perfusion with filtered sea-water; B — perfusion with artificial sea-water without Ca^{2+}; C — perfusion with artificial sea water with Ca^{2+}. Drug in the bath — 1 minute.

b) *Atropine* — blocks the effect of ACh, depending on the concentration of both, the ester and the alcaloid. On washing the preparation the organ reacts by a series of normal spontaneous contra-

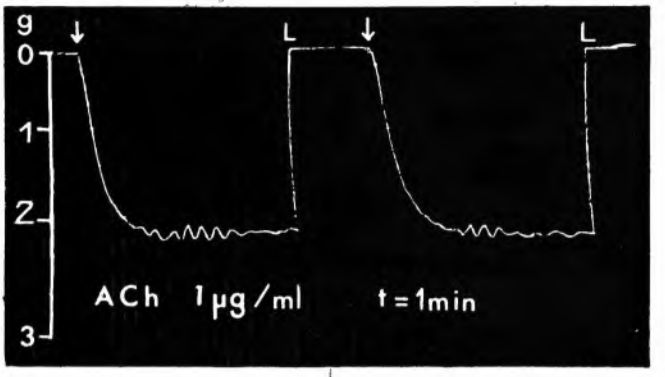


Fig. 3 — Isométric contraction of the oesophagus of *A. brasiliana* stimulated by ACh.

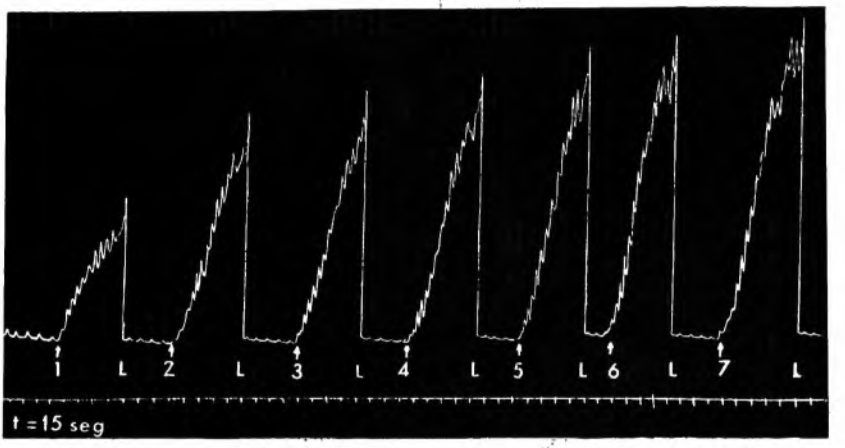


Fig. 4 — Stimulation of the oesophagus of *A. brasiliana*
1 — 0.65 µg/ml ACh; 2 — 1.3 µg/ml ACh; 3 — 1.95 µg/ml ACh; 4 — 2.6 µg/ml ACh; 5 — 3,25 µg/ml ACh; 6 — 3.90 µg/ml ACh; 7 — 4.55 µg/ml ACh.

ctions. A preparation sensitive to $2x10^{6}$ ACh is blocked by $6x10^{-7}$ atropine (Fig. 5). Isometric contraction shows that the tension is between 2 and 3 g when the muscle is stimulated by ACh. After atropinisation the tension decreases to 1 g (Fig. 6).

An interesting effect of Atropine is that of blocking the spontaneous contractions under influence of ACh (Fig. 6).

c) *Nicotine* — Using 2 μg per ml of Nicotine the oesophagus reacts with a very strong contraction and does not relax even washed several times. Unless 20-30 minutes are elapsed the relaxaton time is of 20 or 30 minutes after washing.

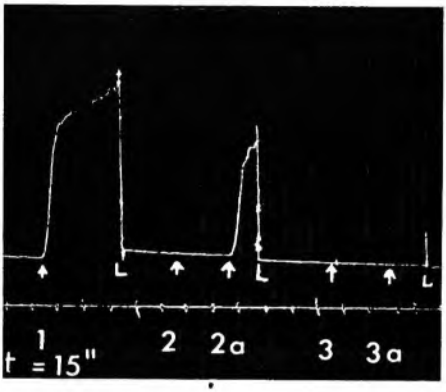


Fig. 5 — Contraction of the oesophagus of *A. brasiliana*.
1 — 2μ/ml ACh; 2 — 0,6 μg/ml Atropine; 2a = 2 μg/ml ACh; 3 = 0,6 μg/ml Atropine; 3a = 2 μg/ml ACh; L = washing.

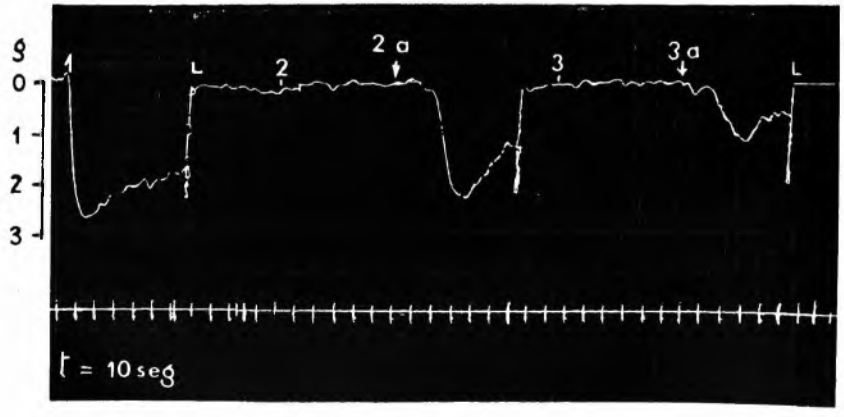


Fig. 6 — Isometric contraction of the oesophagus of *A. brasiliana*. 1 — ACh μg/ml; 2 — Atropine 0.2 μg/ml; 2a — added 10 μg/ml ACh; 3 — Atropine 0,5 μg/ml; 3a — added 10 μg/ACh.

d) *Serotonine* — (5 - Hydroxytryptamine) — The influence of the 5-hidroxytryptamine varies according to concentration. Weak dosis of the drug ($7x10^{-5}$) provokes strong contractions (Fig 7-8) but the spontaneous movements are more intense.

3. *Effects of Calcium ions*:

In these preliminary experiments only Ca^{2+} were tested. Absence of Ca^{2+} in the perfusion fluid (artificial sea water) reduces intensively the effect of ACh. After adding Ca the muscles behave as normally. Fig. 2 A-C indicates the results, when the preparation is stimulated by ACh (2 µg/ml) and perfused with seawater withouth Ca^{2+}. Stimulation by ACh at the same concentration, in such a condition determines a decrease of contractions and the spontaneous movements are strongly reduced (Fig. 2B). If Ca^{2+} ions are then added to the fluid and the muscle excited again, the

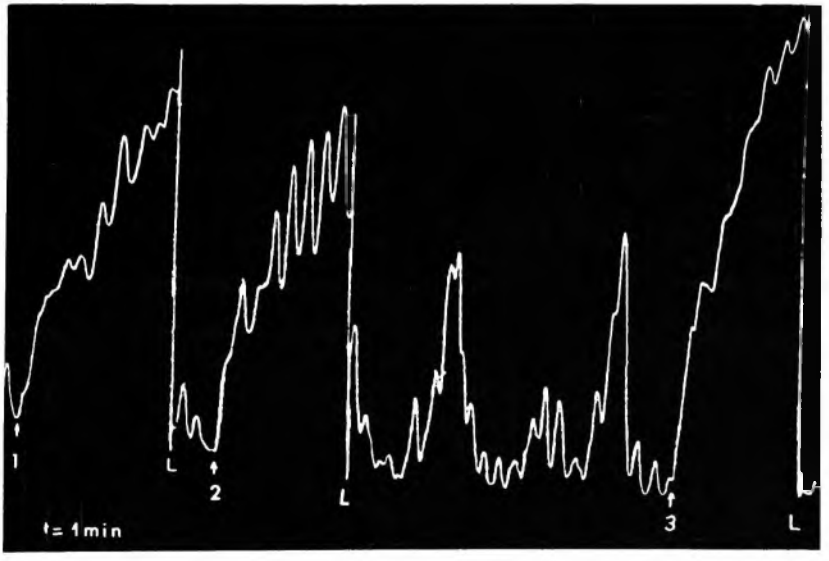


Fig. 7 — Reaction of the oesophagus of *A. brasiliana* to Serotonine. 1 = 70 µg/ml; 2 = 60; 3 = 120.

previous effect is repeated (Fig. 2C). These results are also obtained with the isometric contraction (Fig. 9). If the preparation are trea-

ted by Serotonine in the same way there are also a decreasing of contractions, and recovering of the muscle when small amount of Ca^{2+} is added to the perfusion fluid (Fig. 10).

4. *Differences between the regions of the oesophagus*

As have been said, when the total organ is used the reactions to drugs mentioned above are normal, but if only the anterior third of the organ is perfused, the preparation does not react to those drugs and does not present spontaneous movements. In some cases only

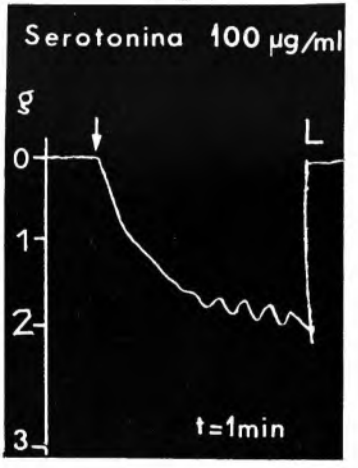


Fig. 8 — Isometric contraction of the oesophagus of *A. brasiliana*.

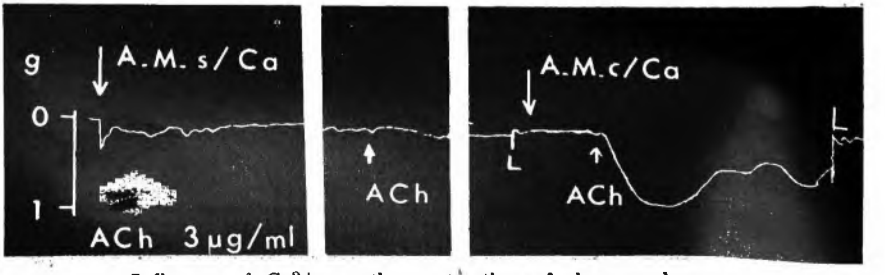


Fig. 9 — Influence of Ca^{2+} on the contraction of the oesophagus of *A. brasiliana* stimulated by ACh. Isometric contraction.
A — artificial sea-water without Ca^{2+}; B — Idem; C — Ions Ca^{2+} added to the perfusion fluid.

very slight contraction can be obtained in such conditions. On the other hand the 2/3 of the organ have strong spontaneous contractions (Fig. 11) and have the same sensitiveness to ACh.

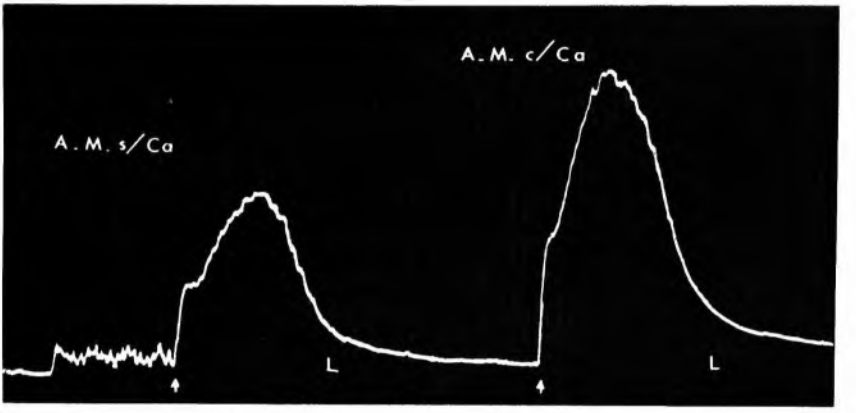


Fig. 10 — Influence of ions Ca^{2+} on the contraction of the oesophagus of *A. brasiliana* treated by Serotonine 60 μg/ml. — artificial sea-water without Ca^{2+}; K — ions Ca^{2+} added. L = Washing.

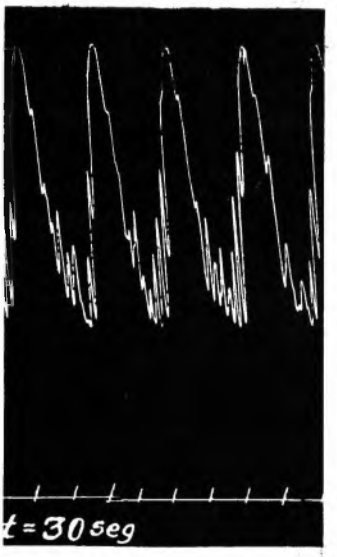


Fig. 11 — Spontaneous contractions of the 2/3 posterior of the oesophagus of *A. brasiliana*.

The explanation of this fact is probably related to the innervation of the anterior third of the organ, which is connected with the cerebral ganglia. The posterior part of the oesophagus is innervated by branches originated from the pedal ganglia (Eales 1927).

4.

DISCUSSION AND RESULTS

No information was obtained from the litterature on the physiology of visceral muscles of Opistobranchs.

Smooth muscles of the oesophagus, chiefly the 2/3 posterior react to ACh in the same way as the thin intestine of Mammals, such as guineapig ileum, rabbit or rat taenia coli.

As it is known no few smooth muscles as, for example, those of the longitudinal body walls of *Holothuria grisea* (Sawaya and Ancona Lopez 1939; Sawaya 1962) present the interesting phenomenon of the influence of atropine, which at dosis by dosis, does not block the effect of ACh. On the contrary, the oesophagus of *Aplysia brasiliana* behave in the same way of Vertebrates smooth muscle that is, contraction of total organ is blocked if the muscle is previously treated by Atropine.

As it has been seen the oesophagus of this Opistobranch is sensitive to the lack of Ca^{2+} in the perfusion bath. The influence of ACh is depending upon the amount of those ions. This fact might indicate that the muscular membrane will be depolarized by other ions, chiefly by K^{+}. The lacking of Ca^{2+} would induce a strong influence of K^{+} which does not allow the interference of ACh. On the other hand it is possible to admit that ACh acts directly on the smooth muscle fiber, and would not have anything to do with the muscular membrane permeability.

As Schild pointed out (1964, p. 99) the calcium requirement at equilibrium for the ACh response of depolarized smooth muscle varies according to the preparation. Probably this will be the case of the oesophagus of *Aplysia*. When the organ is perfused with artificial sea water deprived of Ca^{2+} the muscle practically does not

react to ACh even when strong dosis are used ($2x10^{-6}$) but small amount of Ca^{2+} added to the bath is sufficient to maintain a substantial ACh response for an indefinite period.

Schild (1. c. p. 99) raises the question whether the decline of the ACh effect in the absence of calcium can be attributed directly to the loss of extracellular calcium or whether the essential feature is a reduction of a bound fraction in dynamic equilibrium with extracellular calcium. The rapidness of the rate of decline of the ACh response suggests the first statement, but these results are preliminary and must be confirmed. As the author said, possible each ACh stimulus liberates a quantity of calcium, but whether the calcium came from the extracellular fluid or from a bound store, it not yet clear.

The oesophagus of *Aplysia* requires calcium for contractions.

5.

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