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CONTRACTILE RESPONSES IN THE PRESENCE OF ELECTRON DONORS AND ACCEPTORS

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SUMMARY

Serotonin, a good electron donor, was found to have little or no effect on the proboscis of Phascolosoma. The preparation, however, could be sensitized to serotonin by prior application of electron acceptors (sevron blue, acridine red or riboflavin).

Similarly, acceptors (naphthoquinone or iodine) sensitized striated muscle to a donor (iodide), producing contracture.

It is suggested that electron-transfer reactions could be involved in the contractile responses.

INTRODUCTION

Observations on electronic properties of numerous substances of biological importance, have led SZENT-GYÖRGYI to suggest that charge transfer reactions may be of widespread biological importance[1]. Many drugs are remarkably good donors[1–4] and it is also conceivable that the transfer of single electrons may be involved in muscular contraction.

On the basis of these concepts, experiments were performed on smooth and striated muscle to study the effects of combining certain electron donors with acceptors. The donors (serotonin and iodide) were chosen because of their known effects on muscle while the acceptors (sevron blue, acridine red, riboflavin, naphthoquinone and iodine) were selected purely for their acceptor properties.

MATERIALS AND METHODS

The smooth muscle preparation was the isolated proboscis of Phascolosoma (*Golfingia gouldii*). The tentacles and cerebral ganglion were severed and the ventral nerve cord was stripped in most instances, leaving the torn segmental nerve fibers. This preparation will be referred to as "dernervated". In some cases the ventral nerve cord was left intact. A segment of the proboscis, about 2 cm long, was immersed in a chamber with oxygenated sea water; in some experiments, however, artificial sea water was used with Tris buffer, pH 7.8–8.0. Experiments were done at room temperature (20–23°) during summer and winter months. The isometric tension was recorded with a strain gauge (FT .02, Grass). Initially, the proboscis was stretched to attain a resting tension of about 1 g. The acceptors used were sevron blue, acridine red and riboflavin-5'-phosphate. Solutions were made with sea water. The pH of the serotonin–creatinine sulphate solution was adjusted to 7.8–8.0. 0.1-ml quantities of the solutions were added to the 10 ml of bathing sea water.

The striated muscle was the sartorius of the frog (*Rana pipiens*). The muscle was immersed in oxygenated Ringer's solution (115 m*M* NaCl, 4.0 m*M* KCl, 2.1 m*M* $CaCl_2$, 1.25 m*M* Na_2HPO_4, 0.5 m*M* NaH_2PO_4; pH 7.1). Experiments were done at room temperature during summer months. Isometric tension was recorded with a strain gauge (FT 10, Grass). The muscle was stimulated by massive transverse shocks[5] with a Grass Stimulator (S4A). The electron donor used here was iodide and the acceptors were iodine and 1,2-naphthoquinone-4-sulfonic acid sodium salt. To apply the iodide, the NaCl in Ringer's solution was replaced by NaI. The acceptors were dissolved in Ringer's solution and 0.5-ml quantities were added to the 25 ml of Ringer's solution in the chamber.

All concentrations given under RESULTS will be the final ones after mixing. Mixing was ensured by bubbling oxygen.

The chemicals used were all commercial preparations and were not purified further. Data on the energy levels of sevron blue and acridine red were supplied by PULLMAN AND PULLMAN[6]. For information on the electronic properties of the remaining substances used see ref. 1.

RESULTS

Smooth muscle

Behaviour of the isolated proboscis: With the ventral nerve cord intact, the proboscis contracted spontaneously. Rhythmic contraction occurred usually at a frequency of 20–40/h with a force of about 5–15 g. The rate and force varied with each preparation. When the nerve cords were stripped, contractions ceased in all cases. Hence, it is assumed there are cell bodies situated in the nerve cords (as in annelids[7]) which act as centers for rhythmic activity.

"Denervated" proboscis: A summary of the main findings on the quiescent "denervated" proboscis appears in Table I.

Serotonin (10^{-6}–10^{-4} *M*) had either no effect or induced only a single phasic contraction within 30 sec after application.

Sevron blue (10^{-4} *M*) alone produced either no response or had a relatively minor effect causing either a single phasic contraction on application or phasic contractions at infrequent intervals while the preparation was immersed in the dye for 0.5–3 h.

TABLE I

SUMMARY OF RESULTS ON "DENERVATED" PROBOSCIS

Treatment	*No. of preparations*	*Incidence of different types of responses*			
		No response	*Single phasic contraction*	*Few phasic contractions*	*Marked phasic and tonic contractions*
Serotonin	34	21	13	—	—
Sevron blue	28	10	9	9	—
Sevron blue + Serotonin	36	2	4	10	20
Acridine red	15	12	—	3	
Acridine red + Serotomin	15	2	6	7	—
Riboflavin	8	8	—	—	—
Riboflavin + Serotonin	8	5	—	—	3*

* Mainly tonic contraction with small superimposed phasic contractions.

Combined effect of sevron blue and serotonin: On adding sevron blue (10^{-4} *M*) 5–15 min after the application of the serotonin, there was a marked response after 0.5 to 1.5 h immersion. There were strong phasic contractions associated initially with an increased tone. It was also apparent that a certain time interval was required for the sevron blue to have its effect. Hence, a number of preparations were first soaked in sevron blue for a period of about 3 h. On subsequent addition of serotonin, immediate marked contractile responses were obtained (Fig. 1). On application of higher concentrations of sevron blue ($2 \cdot 10^{-4}$ or $3 \cdot 10^{-4}$ *M*) for shorter periods (10–30 min) serotonin produced similar results.

Because of the known photosensitization produced by a number of dyes in muscle[8], a number of experiments were performed with the preparation enclosed in a black box; this procedure did not alter the results.

In view of the known relaxing effect of serotonin in tonically contracted molluscan muscle[9], mention should be made of an incidental finding. In three preparations the tone increased gradually and relaxation was induced, as in molluscan muscle, by serotonin. But, of particular significance to this study, two of the preparations had been soaking in sevron blue and the relaxation was followed by marked phasic contractions. By contrast, the other preparation was soaked in sea water without sevron blue and here the muscle remained quiescent after the serotonin-induced relaxation.

Effect of acridine red and serotonin: Addition of serotonin (10^{-6}–10^{-4} *M*) to preparations soaking in acridine red (10^{-4} *M*) for 3 h, resulted in phasic contractions which were more marked than those produced by acridine red alone. They were, however, less frequent and forceful than those with sevron blue.

Effect of FMN and serotonin: FMN had no effect on preparations soaking up to 3 h. The subsequent addition of serotonin produced tonic contraction with superimposed small phasic contractions in a small number of instances.

Spontaneously contracting proboscis: As already mentioned, the proboscis contracted spontaneously if the nerve cord was not stripped. The spontaneous contraction became progressively weaker and less frequent, stopping after 36 h. Using an approach similar to that of BÜLBRING AND BURNS[10] in their studies on acetylcholine and heart muscle, an attempt was made to restore activity by the application of combinations of serotonin and sevron blue. Recovery could not be obtained after complete cessation

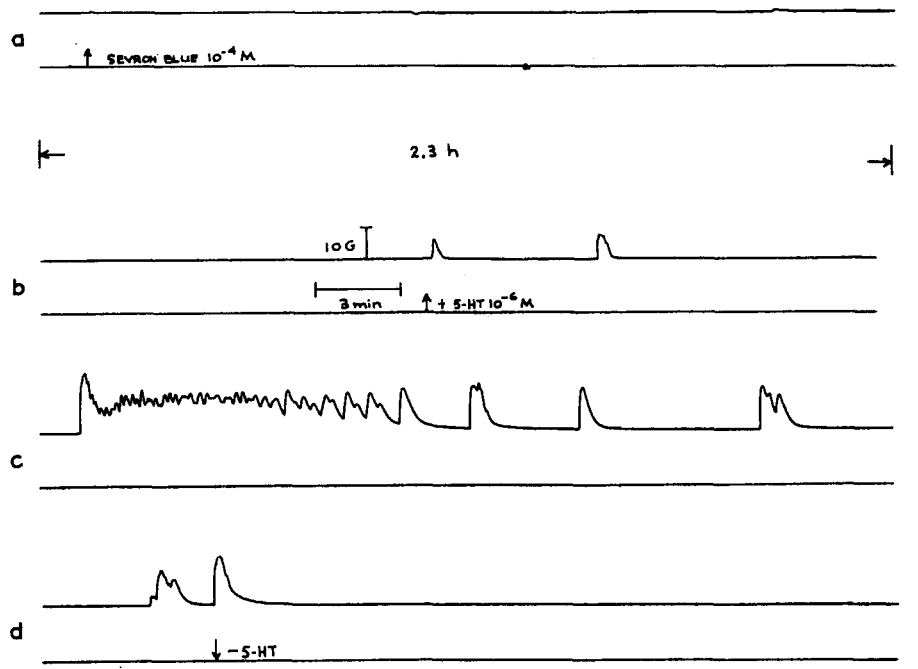

Fig. 1. Tension recording of isolated proboscis. Continuous recordings are depicted from above downwards. The upper line of each double row (a,b,c,d) is the tension recording and the lower one is the base line. Recordings during a period of 2.3 h (between rows a and b), are omitted; during this period there were only a few very small phasic contractions. The addition of sevron blue (row a) had hardly any effect on the preparation while soaking up to 3 hours. Addition of serotonin (5-HT) (row b) led to an immediate small phasic contraction. This was followed soon after by an increased tone with a train of superimposed phasic contractions (row c) and when the tone decreased there were continued phasic contractions until the 5-HT was "washed" out (row d). "Washing out" of 5-HT did not always have an immediate effect. "Washing out" of sevron blue usually stopped contraction even though the preparation remained "stained".

of activity but if the serotonin and sevron blue were added when the preparation was still weakly active, considerable augmentation of the rate and force of contraction was produced. Thus again, the combination of an electron donor and acceptor promoted, in some way, cyclical activity.

Striated muscle

Iodide has numerous effects on striated muscle[5,11] but does not induce contraction. In this investigation, iodide induced contracture of the sartorius, provided the muscle was pretreated with an electron-acceptor (naphthoquinone (10^{-3} *M*) for about 1.5 h, or with iodine ($0.26–1.3 \cdot 10^{4}$ *M*) for about 0.5 h. The contracture relaxed gradually. There was also a decreased response to electrical stimulation and subsequent loss of reactivity; this was irreversible. Each substance alone caused no contractile response. But a number of preparations which were soaked in naphthoquinone for a longer period (over 4 h) developed a slight gradual contracture and loss of response to electrical stimulation.

Mention should also be made that tonic contraction was induced in the smooth muscle preparation by combining iodine or sevron blue with iodide. On the other hand naphthoquinone alone produced an immediate tonic contraction.

DISCUSSION

Little is known on the mechanism of action of serotonin. In the case of smooth muscle GADDUM postulated the existence of specific receptor sites for serotonin[12] and WOOLEY reported on the extraction of part of a serotonin receptor[13]. It is thought that a serotonin-receptor complex enhances the passage of Ca^{2+}, which in turn is involved in producing contraction[14]. In the present investigation a mechanism of action for serotonin was considered in terms of charge transfer processes. Thus, when serotonin was found to have little or no effect on the smooth muscle preparation it was thought that the application of a suitable electron acceptor in combination with serotonin might promote charge transfer processes; this in turn could induce or enhance contractile responses. These responses indeed occurred when serotonin was combined with acceptors. Hence, the results are in keeping with the idea of serotonin acting through charge transfer processes on smooth muscle.

Similarly, the results on the striated and smooth muscle support the suggestion that the iodide effect also involved charge transfer processes.

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