TRANSMURAL ELECTRICAL STIMULATION-INDUCED RELAXATION OF THE GUINEA-PIG GALL BLADDER

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The responses of isolated gall bladder to transmural electrical stimulation with rectangular pulses (50 volt, 0.5 ms) of 30 Hz for 10 s at intervals of 30 min were observed for 5 to 6 hours continuously and compared for different values of resting tonus in Krebs solution at 27°C and 37°C. In 27°C Krebs solution, the resting tonus increased gradually with time and became stable at 0.56 ± 0.07 g (mean $\pm$ S.D.) after 5 h. In 37°C Krebs solution, the resting tonus increased gradually with time and became stable at 1.48 ± 0.49 g (mean $\pm$ S.D.) to 1.5 ± 0.19 g (mean $\pm$ S.D.) after 5 h. Monophasic contraction was observed when the resting tonus was lower than 1.49 ± 0.15 g (mean $\pm$ S.D.), but above this value, biphasic response consisting of contraction and relaxation was observed. Both monophasic and biphasic responses were blocked by atropine and tetrodotoxin. Physostigmine enhanced monophasic contraction, increased the duration of contraction, and abolished the relaxation of biphasic contractile response. The results suggest that contractile response of isolated guinea-pig gall bladder to transmural electrical stimulation was directly induced by acetylcholine released from postganglionic cholinergic fibers. Transmural electrical stimulation with rectangular pulses (50 volt, 0.5 ms) at 10, 20, 30 or 40 Hz for 10 s induced biphasic response in which the contraction varied with frequency, peaking at about 30 Hz, while the relaxation was almost constant. Biphasic relaxation was reduced or inhibited by 2,4-dinitrophenol, ouabain, or K⁺-free solution. This suggests that the relaxation phase of the biphasic response induced by transmural electrical stimulation was an active process.

Keywords—gall bladder; biphasic response; transmural electrical stimulation-induced relaxation; 2,4-dinitrophenol; ouabain; K⁺-free

INTRODUCTION

Transmural electrical stimulation of the guinea-pig gall bladder has been reported to evoke contraction.¹⁾ When guinea-pig gall bladder *in vitro* was stimulated, the response was biphasic consisting of contraction and relaxation. The relaxation was concluded to be inhibited by β -blocker so that it seemed to be an adrenergic inhibitory nerve-mediated relaxation. This was observed mainly when tonus was high, or during spontaneous contraction.²⁾ It has been reported that the relaxation appearing in response to stimulation of the intramural nerves occurs in the presence of atropine. This relaxation is not inhibited by α or β -blockers, but is inhibited by tetrodotoxin. It was thus concluded that the relaxation was mediated by non-adrenergic inhibitory

nerves. The authors said that this was most easily demonstrated when the tonus of the preparation was high.³⁾

There thus appears to be a relation between the activity of the inhibitory neurons and the tonus of the gall bladder. It might also be possible that the tonus of the tissue itself induces relaxation myogenically. The present study was undertaken to investigate quantitative relations between the appearance of transmural electrical stimulation-induced relaxation and spontaneous changes in the tonus of the muscle. The effects of various pharmacological agents on these relations were also investigated.

MATERIALS AND METHODS

Guinea-pigs (250–300 g) were killed by a

blow on the head and exsanguinated. The gall bladder was removed and divided into two approximately equal parts, longitudinally. One of these pieces was used in an experiment. The preparation was mounted in an organ bath which was filled with Krebs solution. As shown in Fig. 1, the fundus of the gall bladder was fixed with pins and the cervix end was connected with a silk thread to a force transducer. The mechanical responses of the gall bladder were recorded isotonicly under a load of 1 g. Two 5 mm $\times$ 5 mm silver plates coated with silver-silver chloride were used as stimulus electrodes. The electrodes were placed, one on the serosal side and the other on the mucosal side of the tissue and connected to a stimulator. The space between the tissue and each electrode was approximately 1 mm. Tissue was stimulated transmurally with rectangular pulses (50 volt, 0.5 ms, 30 Hz) for periods of 10 s. The preparation was bathed in a Krebs solution having the following composition (mM): 133.5 NaCl, 4.7 KCl, 2.5 CaCl_2 , 0.1 MgCl_2 , 1.4 NaH_2PO_4 , 16.3 NaHCO_3 and 7.8 glucose, perfused with 95% O_2 + 5% CO_2 , and having pH 7.4.

FIG. 1. Scheme of Experimental Arrangement

Tissue from gall bladders was divided into about equal parts longitudinally. Upper view: fundus end of gall bladder fixed with pins ($\bullet$), and cervix end connected with silk thread to transducer. Position of stimulating electrodes shown.

Changes in the resting tonus of the muscle, which increased spontaneously in 27°C and 37°C Krebs solution, were observed. The experiment was started 2 hours after placing the gall bladder in an organ bath. Changes in the resting tonus of the tissue and responses to transmural electrical stimulation were recorded and calculated over a period of 5 to 6 hours at intervals of 30 minutes. In the 2 hour period before beginning the experiment, resting tonus decreased slightly, but never increased.

The drugs used were: atropine sulfate (Iwaki Seiyaku Co.), 1×10^{-6} g/ml; tetrodotoxin (Sankyo Co.), 1×10^{-7} g/ml; physostigmine sulfate (Tokyo Kasei Kogyo Co.), 1×10^{-7} g/ml; guanethidine sulfate (Tokyo Kasei Kogyo Co.), 1×10^{-6} g/ml; hexamethonium chloride (Tokyo Kasei Kogyo Co.), 5×10^{-5} g/ml; 2,4-dinitrophenol (Tokyo Kasei Kogyo Co.), 1×10^{-6} g/ml; ouabain (Merck), 1×10^{-6} g/ml. The concentrations indicated were the final values in the organ bath.

RESULTS

Change of Resting Tonus with Time and Relaxation induced by Transmural Electrical Stimulation

FIG. 2. Time Courses of Change of Resting Tonus in Response to Transmural Electrical Stimulation

Time 0, beginning of experiment (2 h after placing gall bladder in bath). A: Changes in 27°C Krebs solution. Number over each response indicates time in hours after beginning of test. B: Changes in 37°C Krebs solution. Notations, same as in A. Biphasic response appeared at 4.5 h in this experiment. Initial resting tonus shown by dotted line.

As shown in Fig. 2:A, spontaneous contraction of the tissue was seldom observed, or was very weak in 27°C Krebs solution, and the resting tonus changed very little for the first half to one hour of the experiment. Response to transmural electrical stimulation was also very weak during that time. As time passed, the resting tonus gradually increased and transmural electrical stimulation produced progressively greater

FIG. 3. Time Courses of Resting Tonus Change

0 g: tonus at beginning of experiment. 0 h: time at beginning of experiment after 2 h of preparation equilibration. ○: tonus in 27°C Krebs solution. ●: tonus in 37°C Krebs solution. Each point: mean $\pm$ S.D., $n=10$. Dotted zone: region (mean $\pm$ S.D., 1.49 ± 0.15 g, $n=30$) in which biphasic responses were observed.

FIG. 4. Relations between Contraction and Relaxation in Biphasic Responses induced by Transmural Electrical Stimulation

Preparation stimulated with rectangular pulses (50 volt, 0.5 ms) at 5, 10, 20, 30 and 40 Hz, as indicated, for periods of 10 s.

monophasic contractions. The contractions induced by the stimuli achieved their maximum amplitude in three to four hours, and remained at approximately the same level thereafter. The resting tonus reached a steady state at 0.56 ± 0.07 g in 4 to 5 hours after the beginning of the experiment (Fig. 2:A, Fig. 3). The transmural electrical stimulation-induced relaxation was never observed at any tonus level which was reached in this Krebs solution.

On the other hand, in 37°C Krebs solution, four effects were observed: 1) Tonus reached a level higher than that in 27°C Krebs solution. 2) Spontaneous contractions appeared. 3) Con-

FIG. 5. Responses of Guinea-pig Gall Bladder in 27°C Krebs Solution to Transmural Electrical Stimuli applied at Dots Beneath each Tracing

1: effect of atropine (Atr), 1×10^{-6} g/ml., 2: effect of tetrodotoxin (TTX), 1×10^{-7} g/ml., 3: effect of physostigmine (Physo), 1×10^{-7} g/ml., 4: effect of guanethidine (Gua), 1×10^{-6} g/ml., 5: effect of hexamethonium (Hexa), 5×10^{-5} g/ml. Each agent was applied at arrow.

traction in response to stimulation was greater than in 27°C Krebs solution. 4) Post-contraction relaxation, the second phase of a biphasic response, appeared (Fig. 2:B). The fourth effect appeared when the resting tonus approached its maximum value. The increased resting tonus (effect 1), above) is illustrated in Fig. 3. This became somewhat stable between 1.48 and 1.5 g. Moreover, as indicated in Fig. 3, the biphasic responses were observed only after the resting tonus reached 1.49 ± 0.15 g (mean $\pm$ S.D., $n=30$).

Relation between Amplitude of Contraction and Depth of Relaxation in Biphasic Response induced by Transmural Electrical Stimulation

Using preparations in which tonus was sufficient to observe the biphasic response to transmural electrical stimulation, the effects of stimulating frequency were observed in order to determine whether the depth of relaxation in the biphasic response depended on the amplitude of

contraction which preceded the relaxation. The preparation was stimulated with rectangular pulses (50 volt, 0.5 ms) at 5, 10, 20, 30 and 40 Hz for periods of 10 s. As the stimulus frequency increased, the amplitude of contraction in the biphasic response also increased until it reached a maximum at about 20–30 Hz and then decreased slightly at 40 Hz (Fig. 4). The depth of relaxation was shallow at 5 Hz, increased to a high value at 10 Hz, and then remained nearly constant at the high level for all higher frequencies up to 40 Hz (Fig. 4).

Pharmacological Effects on Preparation Responses to Transmural Electrical Stimulation

1) *Analysis of the Monophasic Response*—Atropine (1×10^{-6} g/ml) or tetrodotoxin (1×10^{-7} g/ml) prevented the monophasic contraction induced by transmural electrical stimulation, while physostigmine (1×10^{-7} g/ml) enhanced it. Guanethidine (1×10^{-6} g/ml) did not affect the contractile response to transmural

FIG. 6. Responses of Guinea-pig Gall Bladder in 37°C Krebs Solution

Experiments were conducted while resting tonuses were lower than 1.49 ± 0.15 g. In all tracings, transmural stimuli at dots beneath each tracing. 1: response of gall bladder to transmural electrical stimulation, no drug., 2: after atropine (Atr), 1×10^{-6} g/ml., 3: after tetrodotoxin (TTX), 1×10^{-7} g/ml., 4: after physostigmine (Physo), 1×10^{-7} g/ml. Each agent was applied at arrow.

FIG. 7. Responses of Guinea-pig Gall Bladder in 37°C Krebs Solution

Experiments were conducted while resting tonuses were at 1.49 ± 0.15 g or higher. In all tracings, transmural stimuli at dots beneath each tracing. 1: response of gall bladder to transmural electrical stimulation, no drug., 2: after atropine (Atr), 1×10^{-6} g/ml., 3: after tetrodotoxin (TTX), 1×10^{-7} g/ml., 4: after guanethidine (Gua), 1×10^{-6} g/ml., 5: after physostigmine (Physo), 1×10^{-7} g/ml. Each agent was applied at arrow.

electrical stimulation, while hexamethonium (5×10^{-5} g/ml) reduced it. These results are shown in Fig. 5 and 6.

2) *Analysis of the Biphasic Response* — Both the contraction and relaxation phases of the biphasic response produced by transmural electrical stimulation, as shown in Fig. 7, were inhibited by atropine (1×10^{-6} g/ml) and tetrodotoxin (1×10^{-7} g/ml). The biphasic response was resistant to guanethidine (1×10^{-6} g/ml). The effect of physostigmine (1×10^{-7} g/ml) on the amplitude of contraction was slight. However, at the concentration used, it enhanced the tonus of the preparations, increased the duration of contraction, and abolished the relaxation so that the biphasic response changed to a monophasic response (Fig. 7).

In order to determine if the relaxation in the biphasic response caused by transmural electrical stimulation, depended on an active process, and to determine if it was related to metabolism, the effects of 2,4-dinitrophenol (1×10^{-6} g/ml) and K^+ -free Krebs solution (the KCl was omitted) were observed. The 2,4-dinitrophenol reduced the resting tonus slightly. This concentration of 2,4-dinitrophenol remarkably inhibited the relaxation in the biphasic response produced by transmural electrical stimulation which was applied 30 min to 40 min after the 2,4-dinitrophenol was added to the organ bath (Fig. 8:1). However, it had almost no effect on the contraction in four cases and showed only a very slight inhibitory effect in three others. Two effects of ouabain (1×10^{-6} g/ml) were observed. In five trials a large, rapid increase in tonus was observed while in two other trials the increase in tonus was much smaller (Fig. 8:3). However, in all cases, the relaxation of the biphasic response which was produced by transmural electrical stimulation was abolished or reduced considerably in the presence of ouabain (Fig. 8:2, 3). Two different response were also observed with K^+ -free Krebs solution. In four out of six trials there was a rapid rise of tension which was maintained for some time and then gradually declined (Fig. 8:4). In the other two cases, only phasic contraction occurred (Fig.

8:5). However, again in both cases, the relaxation of the biphasic response which was caused by transmural electrical stimulation was inhibited or remarkably reduced (Fig. 8:4, 5).

DISCUSSION

Monophasic response (contraction) to transmural electrical stimulation was observed in 27°C and 37°C Krebs solutions. However, the contraction-relaxation response was seen only in 37°C Krebs solution and then only at high resting tonus of 1.49 ± 0.15 g or more. Thus the biphasic response may not depend on temperature, but

FIG. 8. *Effects of 2,4-Dinitrophenol, Ouabain and K^+ -free on Biphasic Responses*

1: effect of 2,4-dinitrophenol (DNP), 1×10^{-6} g/ml., 2—3: effect of ouabain (Oua), 1×10^{-6} g/ml., 4—5: effect of K^+ -free (K-free). Each pair, 2—3, and 4—5, shows two different responses in different preparations subjected to same treatment. After each agent was applied, as shown in 1—5, relaxation in biphasic response was reduced or inhibited.

might depend on the magnitude of the resting tonus in the gall bladder. The relaxation phase of the biphasic response was probably not closely related to the amplitude of the contraction which preceded the relaxation, since the relaxation increased to a high value at 10 Hz while the maximum contraction amplitude occurred at about 30 Hz (Fig. 4).

The monophasic contraction was inhibited by atropine and tetrodotoxin, enhanced by physostigmine, and was unaffected by guanethidine. Thus, it was probably mediated by intramural cholinergic excitatory nerves. The reduction of the monophasic contraction by hexamethonium suggests that the response to transmural electrical stimulation in this experiment was induced partly by stimulation of the intramural ganglion, and partly by nerve terminal stimulation. On the other hand, the biphasic response was almost completely prevented by atropine or tetrodotoxin. Physostigmine enhanced the resting tonus of the tissue and it prolonged the duration of contraction in the biphasic response, while it inhibited the relaxation in the biphasic response. The relaxation phase of the biphasic response was unaffected by guanethidine. Therefore, the intramural cholinergic excitatory nerve also seems to be responsible for the relaxation phase of the biphasic response. The inhibition of relaxation which is produced in the biphasic response by physostigmine may suggest that acetylcholine, in some way prevents the relaxation. This effect would be a result of the inhibition of cholinesterase activity by physostigmine. Yanaura *et al.*²⁾ reported a biphasic response consisting of contraction and relaxation evoked by transmural electrical stimulation. The relaxation phase of this response was abolished by propranolol. They also reported that relaxation was induced by transmural electrical stimulation in the presence of atropine. This relaxation was abolished by tetrodotoxin. Consequently, they suggested the presence of intramural adrenergic inhibitory nerve fibers in the guinea-pig gall bladder. Davison *et al.*³⁾ also demonstrated relaxation induced by transmural electrical stimulation in

the presence of atropine. This relaxation was unaffected by both α and β -blockers, but was prevented by tetrodotoxin. They thus suggested that there were non-adrenergic inhibitory nerve fibers in the guinea-pig gall bladder. Al-Hassani *et al.*⁴⁾ recently observed both α and β -adrenergic responses during transmural electrical stimulation of the guinea-pig gall bladder. In the present report, relaxation was not seen in the presence of atropine. Davison *et al.*³⁾ found it necessary to increase the tonus of the preparation in order to demonstrate the relaxation response. In the present experiment, which was based on investigation of preparation tonus, the existence of intramural inhibitory neurons was not demonstrated. We can not yet explain these differences, but different experimental methods may be contributory. Yanaura *et al.*²⁾ and Davison *et al.*³⁾ used intact gall bladders, but we used sectioned gall bladders.

Two possible mechanisms might explain the relaxation which was observed in the biphasic response to transmural electrical stimulation. In the first, since the relaxation in the biphasic response was observed when the resting tonus of the tissue was high, it might be considered to be a reaction or rebound to release from excessive tension. Since this possibility was not investigated at this time, it can not be discussed here. The other possibility would be an active response in which the relaxation in the biphasic response is quite different from a simple process which is the reverse of muscle contraction. The existence of an active relaxation or response was reported by Singh *et al.*⁵⁾ Yoshida⁶⁾ also found a biphasic response and suggested that the relaxation in the biphasic response was an active relaxation related to metabolism. For strong electrical stimulation, the relaxation did not depend on the amplitude of the preceding contraction (Fig. 4). This may favor the possibility of an active process. Ouabain and K^+ -free solution reduced or inhibited the relaxation in the biphasic response (Fig. 8). When the energy needed for activation of muscle is reduced by 2,4-dinitrophenol, a metabolic inhibitor, the relaxation phase of the biphasic response was sup-

pressed (Fig. 8). It thus seems likely that the relaxation which was induced by transmural stimulation in the present experiment was an active process related to metabolism.

Although potassium-induced relaxation has been reported in a variety of isolated smooth muscle,⁷⁻¹⁷⁾ inhibition by potassium in these smooth muscles was prevented by ouabain and can thus be explained by hyperpolarization of the membrane through activation of the electrogenic sodium pump mediated by Na^+ - K^+ ATPase. The electrogenic sodium pump is inhibited by K^+ -free solution in which KCl is removed from the normal nourishing solution.^{7,10)} As mentioned, the intramural cholinergic excitatory nerve seemed to be responsible for the appearance or relaxation in the biphasic response. Bolton,¹⁸⁾ however, reported that when acetylcholine was washed from guinea-pig terminal ileum following a 10 sec application, the membrane repolarized and then hyperpolarized (after-hyperpolarization) beyond the level which existed prior to application of acetylcholine. No after-hyperpolarization was observed following acetylcholine in potassium-free solution, in sodium deficient solution or in the presence of ouabain. Bolton concluded that application of acetylcholine to this smooth muscle disturbed the sodium and potassium gradients across the membrane in a direction which stimulated the electrogenic sodium pump. The increased electrogenic extrusion of sodium was then responsible for the after-hyperpolarization following the application of acetylcholine. It has been reported by Dlouhá *et al.*¹⁹⁾ that acetylcholine potentiates both the muscle electrogenic ionic pump and the Na^+ , K^+ -ATPase activity of crude membrane fractions. These results support the tentative conclusion from the results reported here, that the relaxation phase of the biphasic response induced by transmural electrical stimulation, which is inhibited by 2,4-dinitrophenol, ouabain and K^+ -free solution, is an active process.

REFERENCES

- 1) W.H.Lee and M.Fujiwara: Effect of catecholamines on the contractile response of isolated guinea-pig gall bladder to transmural and chemical stimulation, *Jiritsushinkei* (Japan), **10**, 102–106 (1973).
- 2) S.Yanaura and S.Ishikawa: Adrenoceptors and autonomic nerve control mechanism in the biliary system, *Nippon Yakurigaku Zasshi*, **71**, 39–51 (1975).
- 3) J.S.Davison, M.Al-Hassani, R.Crowe, and G.Burnstock: The nonadrenergic, inhibitory innervation of the guinea-pig gall bladder, *Pflugers Archiv*, **377**, 43–49 (1978).
- 4) M.AL-Hassani and J.S.Davison: Adrenergic responses to transmural electrical stimulation of the guinea-pig gall bladder, *J. Physiol.* (London), **296**, Proceedings of the physiological society, July, 83 p. (1978).
- 5) I.Singh, S.I.Singh, and N.S.Dhalla: Active relaxation of unstriated muscle produced by epinephrine, *J. Physiol.* (London), **200**, 955–958 (1962).
- 6) T.Yoshida: Contractility and relaxation of the taenia coli of the guinea-pig, *Nippon Seirigaku Zasshi*, **25**, 121–132 (1963).
- 7) G.S.Taylor, D.M.Paton, and E.E.Daniel: Characteristics of electrogenic sodium pumping in rat myometrium, *J. gen. Physiol.*, **56**, 360–375 (1970).
- 8) R.Casteels, G.Droogmans, and H.Hendeickx: Membrane potential of smooth muscle cells in K-free solution, *J. Physiol.* (London), **217**, 281–295 (1971).
- 9) R.Casteels, G.Droogmans, and H.Hendrickx: Electrogenic sodium pump in smooth muscle cells of the guinea-pig's taenia coli, *J. Physiol.* (London), **217**, 297–313 (1971).
- 10) T.Tomita and T.Yamamoto: Effects of removing the external potassium on the smooth muscle of guinea-pig taenia coli, *J. Physiol.* (London), **212**, 851–868 (1971).
- 11) S.Shibata, H.Fukuda, and K.Kurahashi: The inhibitory action of potassium on the mechanical and membrane activities of taenia from guinea pig caecum, *Life Sci.*, **12**, 425–431 (1973).
- 12) T.Ishii and Y.Shimo: Potassium-induced relaxations of the guinea-pig taenia coli, *Arch. int. Pharmacodyn.*, **239**, 36–44 (1979).
- 13) T.B.Bolton: Effects of electrogenic sodium pumping on the membrane potential of longitudinal smooth muscle from terminal ileum of guinea-pig, *J. Physiol.* (London), **228**, 693–712 (1973).
- 14) A.Johns and D.M.Paton: Inhibitory effect of sodium pumping on spontaneous contractility of rabbit myometrium, *Can. J. Physiol. Pharmacol.*, **52**, 786–790 (1974).
- 15) O.Reiner: The role of the electrogenic sodium pump in the potassium relaxation of the rabbit ear artery, *Naunyn-Schmiedeberg's Arch. Pharmacol.*, **303**, 213–220 (1978).
- 16) H.Ozaki, H.Karaki, and N.Urakawa: Possible role of $\text{Na} - \text{Ca}$ exchange mechanism in the contractions

- induced in guinea-pig aorta by potassium free solution and ouabain, *Naunyn-Schmiedeberg's Arch. Pharmacol.*, **304**, 203–209 (1978).
- 17) T.Ishii and Y.Shimo: Potassium-induced relaxation of the rat anococcygeus muscle, *Arch. int. Pharmacodyn.*, **243**, 27–36 (1980).
- 18) T.B.Bolton: The role of electrogenic sodium pumping in the responses of smooth muscle to acetylcholine, *J. Physiol.* (London), **228**, 713–731 (1973).
- 19) H.Dlouha, J.Teisinger, and F.Vyskocil: Activation of membrane Na^+ / K^+ -ATPase of mouse skeletal muscle by acetylcholine and its inhibition by α -Bungarotoxin, curare and atropine, *Pflugers Arch.*, **380**, 101–104 (1979).