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EFFECTS OF PHYSOSTIGMINE ON SMOOTH MUSCLE

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Abstract—The carbamate cholinesterase inhibitor physostigmine (eserine) has been shown to produce effects on the smooth muscle of the toad which cannot be attributed to its anticholinesterase activity. These effects are a depression of excitatory responses to cholinergic nerve stimulation and applied choline esters, and contraction of the muscle. The first of these phenomena has been attributed to an atropinic action of physostigmine at muscarinic receptors on the muscle cell, and the second to enhanced release of acetylcholine from nerve endings.

INTRODUCTION

THE EXCITATORY nerves supplying the bladder of the toad (*Bufo marinus*) appear to be cholinergic in nature, and neostigmine produces potentiation of the contractile responses to both nerve stimulation and applied acetylcholine.[1, 2] The response to physostigmine however is more complex. At low concentrations (up to 10^{-6} g/ml) no effect is observed. This is due to resistance of the tissue cholinesterases to inhibition by physostigmine.[2] At higher concentrations (10^{-5}–10^{-4} g/ml) the tone of the muscle is raised and excitatory responses are depressed. Washout of the bath causes return of the tone to normal and potentiation of excitatory responses. The present paper deals with the mechanisms involved in these actions of physostigmine.

METHODS

The abdominal cavity of a pithed toad was opened in the midline and the ventral body wall deflected laterally. The anterior abdominal vein was cut at its rostral end. The bladder was freed from the dorsal mesentery and the tissue connecting it with the rectum and the ventral body wall was divided. The bladder was then cut about 1 cm above its junction with the cloaca and the two lobes were divided down the midline. Histological examination showed that this procedure gave a preparation which was free of ganglion cells. Each lobe was then mounted in a 50 ml capacity organ bath containing McKenzie's solution,[2] aerated with 95 per cent O_2 + 5 per cent CO_2 and maintained at room temperature (21–25°). The bladder lobe was secured by a tie through its base to a glass J-tube, and isotonic contractions were recorded on a smoked drum via a thread from the apex of the lobe and a frontal point writing lever. Stimulation of nerves in the bladder wall was elicited through two silver ring electrodes having a diameter of 5 mm and a separation of 3 mm. A Grass S5 stimulator was used to deliver square wave pulses of 1 m-sec duration. The frequency of stimulation was submaximal (5–20 pulses/sec dependant on the reactivity of the preparation) for periods of 10 sec. Comparison of the effect of the local anaesthetic dibucaine (cinchocaine) on the responses to stimulation and to applied acetylcholine has shown

that these parameters did not produce direct muscle stimulation. Successive stimulations were separated by a period of at least 6 min.

The following drugs were used: acetylcholine chloride, Roche; atropine sulphate, B.D.H.; carbaminoylcholine chloride (carbachol), B.D.H.; choline chloride, B.D.H.; diisopropylfluorophosphate (D.F.P.), D.S.L.; α-α-dimethyl-ethanolamine 4,4'-*bis*acetophenone dibromide (Hemicholinium-3), Aldrich Chem. Co.; physostigmine (eserine) sulphate, B.D.H.; 3,6 *bis* diethylaminopropoxy pyridazine bis-methiodide (Win 4981), Sterling-Winthrop. All drugs were injected in volumes of 0·1 ml or less, concentrations cited referring to the above salts. Responses to acetylcholine and carbachol were measured over a 30–60 sec contact period separated by intervals of at least 6 min.

Ringer having a pH of 6·3 was prepared by reducing the $NaHCO_3$ content to 0·28 g/l. High potassium ringer was prepared by substituting iso-osmotic quantities of K_2SO_4, KH_2PO_4 and $KHCO_3$ for NaCl, $NaH_2PO_4.2H_2O$ and $NaHCO_3$ respectively.

RESULTS

Effect of physostigmine

Addition of physostigmine (10^{-5}–10^{-4} g/ml) caused alteration of the spontaneous activity of the bladder. In most cases (30/37) a marked rise in tone was seen (Fig. 1a.).

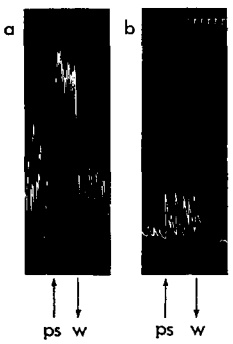

FIG. 1. Effects of physostigmine 10^{-5} g/ml (ps) on two preparations of the toad bladder. Fig. 1 (a) is typical of the type of effect most frequently observed.
Time marker: 1 min. The trace in (b) has been retouched.

In occasional preparations (7/37) the basal tone did not alter, but the amplitude of spontaneous contractions was increased (Fig. 1b). Washing out the bath produced a return in tone and activity to the normal state in 32 out of 37 preparations. In the remainder of this paper either of these effects of physostigmine will be referred to as the "contractile response".

Physostigmine caused a reduction in the response to nervous stimulation by up to 20 per cent, and at concentrations of 5×10^{-5}–10^{-4} g/ml the response to applied acetylcholine (2×10^{-8} g/ml) was also markedly reduced. Following wash out of the bath the responses to nerve stimulation and acetylcholine were potentiated above the control values (Fig. 2).

The response to applied carbachol (5×10^{-8} g/ml) was also reduced by physostigmine. Following wash out of the bath the responses were restored to control value but no potentiation was observed (Fig. 3).

Effects of physostigmine after atropine

Atropine (10^{-8}–10^{-5} g/ml) has only a slight blocking effect on the cholinergic nerves to the toad bladder, although the response to applied choline esters is completely blocked. This situation is typical of bladder preparations[3, 4, 5]. Pretreatment

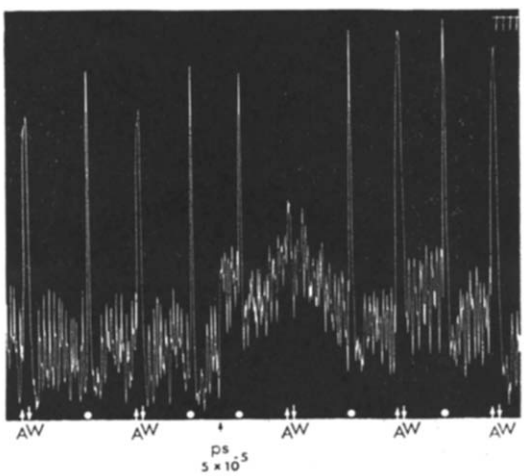

FIG. 2. Effect of physostigmine 5×10^{-5} g/ml (ps) on the responses of the toad bladder to intramural nerve stimulation (white dots) and to applied acetylcholine 2×10^{-8} g/ml (A). Bath washed out at W. Time marker: 1 min.

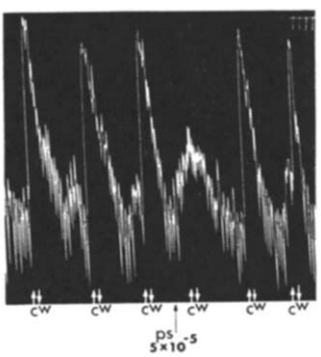

Fig. 3. Effect of physostigmine 5×10^{-5} g/ml (ps) on the response of the toad bladder to carbachol 5×10^{-8} g/ml (C). Bath washed out at W. Time marker: 1 min.

of the preparation for 30 min with atropine (10^{-6}–10^{-5} g/ml) did not prevent the "contractile response" to physostigmine. However responses to nerve stimulation were now potentiated in the presence of physostigmine (Fig. 4). Washing out the bath caused the responses to be gradually reduced towards control values.

Effects of physostigmine after D.F.P.

In some experiments the bladder was incubated with 2×10^{-4} g/ml D.F.P. for 60 min in order to inactivate all tissue cholinesterases. Following this procedure no further potentiation of the responses to nervous stimulation could be obtained with neostigmine (5×10^{-6} g/ml). However physostigmine still produced a "contractile response".

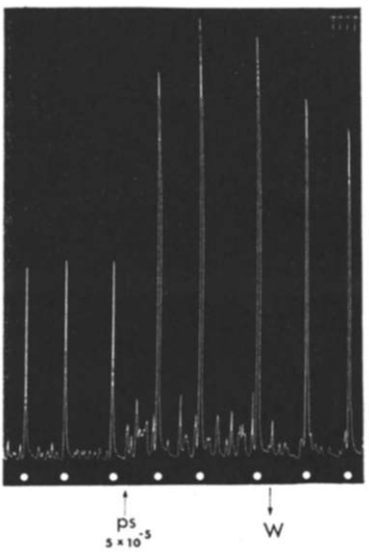

FIG. 4. Effect of physostigmine 5×10^{-5} g/ml (ps) on the response of the toad bladder to intramural nerve stimulation (white dots) following pretreatment of the tissue with atropine (10^{-6} g/ml). Bath washed out at W. Time marker: 1 min.

Effects of physostigmine at pH 6·3

At physiological pH physostigmine exists in a partially tertiary form. Reduction of the pH to 6·3 results in complete conversion of physostigmine to the lipoid-insoluble quarternary state. When immersed in ringer of pH 6·3 the toad bladder showed little or no spontaneous activity. However large responses could still be produced by nerve stimulation or application of acetylcholine. Under these circumstances physostigmine produced a reduction in the response to nerve stimulation which was converted to a potentiation after washing out. The response to acetylcholine (2×10^{-8} g/ml) was sometimes reduced and sometimes potentiated. When reduction was seen, washing always reversed the response to a potentiation. In contrast to bladders bathed in normal McKenzie's solution (pH 7·25) no "contractile response" to physostigmine was observed (Fig. 5).

After replacement of the pH 6·3 ringer with normal McKenzie's solution and equilibrium for 20 min, normal "contractile responses" to physostigmine could be elicited.

Effects of physostigmine in high potassium ringer

Following depolarization of the muscle membrane by replacement of all sodium ions in the ringer with potassium ions no response could be obtained to transmural stimulation, but acetylcholine (2×10^{-8} g/ml) caused some contraction. In these preparations physostigmine caused slight relaxation of the tissue which was reversed

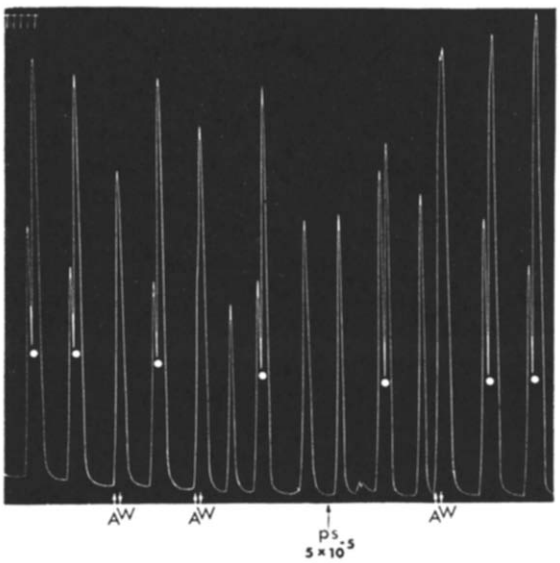

FIG. 5. Effect of physostigmine 5×10^{-5} g/ml (ps) on the responses of the toad bladder to intramural nerve stimulation (white dots) and to applied acetylcholine 2×10^{-8} g/ml (A) in ringer of pH 6·3. Bath washed out at W. Time marker: 1 min. Note that in this particular preparation no initial reduction of the response to acetylcholine was produced by physostigmine.

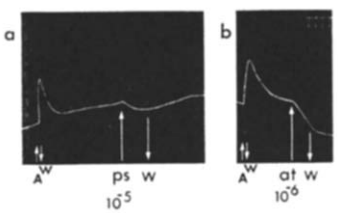

FIG. 6. Effect of physostigmine 5×10^{-5} g/ml (ps) and atropine 10^{-6} gm/ml (at.) on the toad bladder immersed in high potassium ringer. Bath washed out at W. Time marker: 1 min. The traces in this figure have been retouched.

by washing (Fig. 6). Atropine caused a greater relaxation which was not easily reversed by washing. Following replacement of the potassium-rich solution with normal McKenzie's solution and relaxation of the bladder to its normal tone, physostigmine produced a "contractile response".

Effects of physostigmine after hemicholinium or Win 4981*:*

Hemicholinium and Win 4981 have both been shown to deplete pre-synaptic stores of acetylcholine during prolonged high frequency nervous stimulation.[6, 7] Stimulation of the bladder at 50 pulses/sec every 6 min in the presence of hemicholinium or Win 4981 ($3–5 \times 10^{-4}$ g/ml) caused abolition of nervous responses over a period of 60–90 min. Following this procedure the "contractile response" to physostigmine was reduced in amplitude and fell off rapidly. Addition of choline (2×10^{-4} g/ml) to the bath for 15 min caused partial restoration of responses to both nervous stimulation and physostigmine (Fig. 7).

DISCUSSION

It was originally thought[2] that the "contractile response" of the toad bladder to physostigmine might be attributable to an effect on intracellular cholinesterases concerned with control of the inherent motility of the muscle. Similar suggestions have been proposed to explain the contractile effect of physostigmine on the smooth muscle of the guinea-pig trachea[8] and the chick amnion.[9] However the present results do not support this theory.

Treatment of the toad bladder with D.F.P., an irreversible cholinesterase inhibitor which readily crosses membrane barriers, did not affect the response to physostigmine. However, D.F.P. completely inactivated the extracellular cholinesterase, as shown by the fact that the normal potentiating effect of neostigmine on the response to nerve stimulation[2] was not observed after treatment with D.F.P.

Conversely Cuthbert[9] claimed that D.F.P. abolished the effect of physostigmine on the amnion. Complete depolarization of the muscle membrane by immersion in high potassium ringer prevented the "contractile response" to physostigmine, although contractions could still be elicited with acetylcholine. This indicates a site of action for physostigmine exterior to the muscle cell.

Harry[10] observed that physostigmine provoked spontaneous activity of the quiescent guinea-pig ileum, and attributed this effect to direct muscarinic stimulation by physostigmine. The "contractile response" of the toad bladder cannot however be attributed to muscarinic stimulation, as 10^{-5} g/ml atropine did not abolish the effect, although concentrations of atropine as low as 10^{-8} g/ml completely block contractions due to applied acetylcholine.[1]

Complete conversion of physostigmine to the quaternary form by use of ringer at pH 6·3 caused abolition of the "contractile response". In addition, the carbamate neostigmine, which is quaternary at physiological pH, has no contractile effect on the toad bladder.[2] This suggests that the site of action of physostigmine is interior to a membrane barrier. Carlyle[11] reported that low concentrations of physostigmine caused a rise in tone of the guinea-pig tracheal muscle, and that this action could be blocked by atropine and by procedures which inhibited release of neuronal acetylcholine. He attributed the effect of physostigmine to stimulation of release of acetylcholine from nerve endings. A similar action of physostigmine has also been suggested at the sympathetic ganglion.[12] Treatment of the toad bladder with hemicholinium or Win 4981, compounds which have been shown to block neuronal synthesis of acetylcholine,[6, 7] reduced the "contractile response" to physostigmine in parallel to reduction of nervous responses. Choline produced partial restoration of both responses. It seems likely therefore that the increase in tone and spontaneous activity of the

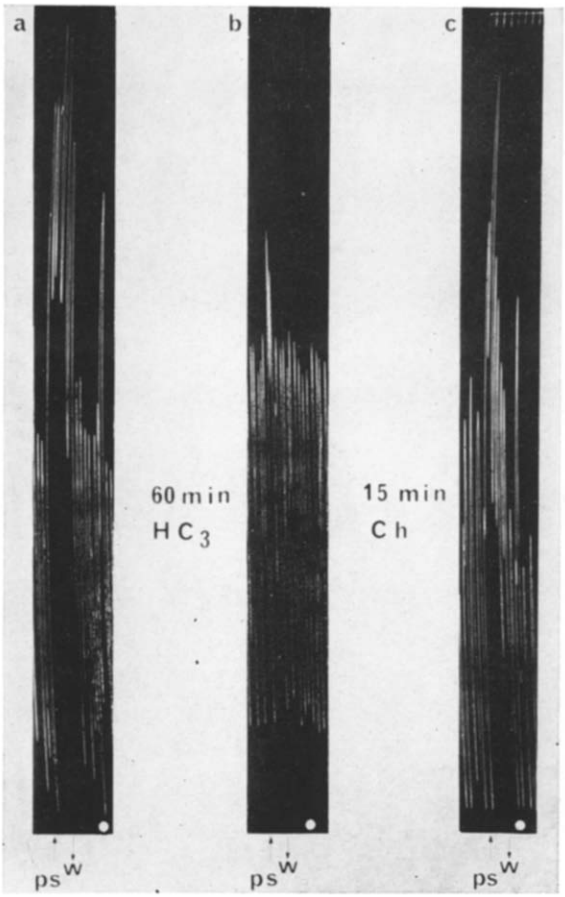

FIG. 7. Effect of physostigmine 5×10^{-5} g/ml (ps) (a) before and (b) after abolition of the response to intramural nerve stimulation (white dots) by prolonged high frequency stimulation in the presence of hemicholinium 5×10^{-4} g/ml. (c): 15 min after the addition of choline 2×10^{-4} g/ml.

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toad bladder seen after addition of physostigmine is due to release of acetylcholine from nerve endings within the tissue. In contrast to the situation in the trachea, atropine was ineffective in blocking the bladder response to physostigmine. However as mentioned above atropine has little blocking action on cholinergic nerves to the bladder.

Physostigmine reduces the responses of the toad bladder to nervous stimulation and to applied acetylcholine and carbachol. This effect has previously been attributed to a depressant effect of physostigmine on the muscle cholinergic receptors.[2] The fact that the reduction of nervous responses by physostigmine is prevented by pretreatment of the tissue with atropine supports this theory.

A depressant effect of physostigmine on cholinergic receptors has been previously reported with amphibians. Hobbiger,[13] Fatt[14] and Porter and deLaLande[15] have all noted a curariform action at the amphibian neuro-muscular junction, while Jung and Barton[16] reported an atropinic effect on the frog heart. The fact that all these claims have resulted from work with amphibian tissues can be attributed to the fact that amphibian cholinesterases are resistant to inhibition by physostigmine and that therefore any depressant effects are unlikely to be over-ridden by cholinergic potentiation. However Tedeschi[17] and Quilliam and Strong[18] have reported that physostigmine can act like atropine on the isolated rabbit auricle, and there seems no reason to doubt that similar effects might be seen in other tissues under suitable circumstances.

A further action of physostigmine not attributable to inhibition of cholinesterases is the direct sensitization of the post junctional membrane to excitatory substances, as suggested for the neuromuscular junction[19] and the ganglionic synapse.[20] In the present study, although responses to nervous stimulation and acetylcholine were potentiated following washout of the atropinic effect, those to the acetylcholine analogue carbachol, which is not hydrolysed by cholinesterase, were not potentiated. It seems therefore that there is no justification for postulating any direct sensitizing effect of physostigmine on the toad bladder.

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