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The effects of beta adrenoceptor blocking agents on the membrane potential and spike generation in the smooth muscle of guinea-pig taenia coli

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Summary

1. The measurement of changes in spike generation and membrane resistance in the guinea-pig taenia coli, using the sucrose gap extracellular recording method, has been shown to be a useful way to demonstrate the intrinsic sympathomimetic activity of the beta adrenoceptor blocking agents.

2. Pronethalol, INPEA, MJ 1999, MJ 1998 and dichloroisoprenaline abolish the spontaneous spike discharge and produce a hyperpolarization of the cell membrane. These sympathomimetic effects were blocked by a mixture of alpha (phentolamine) and beta (propranolol) adrenoceptor blocking agents.

3. Propranolol reduced the frequency of the spontaneous spike potentials but did not raise the resting membrane potential of the taenia coli.

4. When the metabolism of the taenia coli was depressed, all the beta adrenoceptor blocking agents produced a depolarization of the cell membrane and sometimes initiated spike potential activity. The depolarization of the cell membrane was antagonized by superfusing the taenia coli with Krebs solution containing phentolamine. The effects of the beta adrenoceptor blocking agents were not antagonized by the presence of atropine.

5. Experiments performed on the reserpinized taenia coli preparation show that depletion of the stores of catecholamines did not modify the sympathomimetic actions of the beta adrenoceptor blocking agents on the normal or metabolically depressed taenia coli preparation. The sympathomimetic action of the beta blocking agents does not appear to be mediated through the release of catecholamines within the taenia coli.

Introduction

On cardiac muscle, pronethalol (Somani, Hardman & Lum, 1963 ; Donald, Kvale & Shepherd, 1964), INPEA (Meester, Hardman & Baboriak, 1965), MJ 1998 (Stanton, Kirchgessner & Parmenter, 1965) and dichloroisoprenaline (Fleming & Hawkins, 1960 ; Moran & Perkins, 1958) have been reported to exhibit sympathomimetic activity, while propranolol (Shanks, 1966) and MJ 1999 (Stanton *et al.*, 1965) are reported to be devoid of such sympathomimetic activity.

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On smooth muscle preparations, intrinsic sympathomimetic activity may be demonstrated at a cellular level. Bulbring (1960) reported a dual mode of action of sympathomimetic amines on the smooth muscles of the guinea-pig taenia coli. Under physiological conditions, sympathomimetic amines produce a loss of spike potential activity followed by a hyperpolarization of the cell membrane of the taenia coli (Burnstock, 1958b ; Bulbring, 1960). When the metabolism of the taenia coli is depressed, as when the preparation is bathed in modified Krebs solution devoid of glucose, adrenaline restores electrical activity, producing a depolarization of the cell membrane and sometimes initiates spontaneous spike potential activity. This excitatory action of adrenaline can only be demonstrated when the metabolic processes are depressed due to lack of substrate availability (Bulbring, 1960).

The effects of the beta adrenoceptor blocking agents on the spontaneous spike potentials and membrane potentials of the normal and metabolically depressed taenia coli preparations were studied with a view to demonstrating the intrinsic sympathomimetic activity of these agents on a smooth muscle preparation.

Methods

All experiments were performed on smooth muscle strips of taenia coli using the sucrose gap technique (Stamfli, 1954).

Strips of taenia coli 20–25 mm in length were taken from freshly killed guinea-pigs, placed in the apparatus, stretched to their *in situ* length and fixed at this length throughout the experiment. The taenia coli were mounted in the apparatus so that they lay in a horizontal insulated tube containing isotonic sucrose solution (10%w/v) which had a specific resistance of at least 2×10^6 ohm/cm. Each end of the muscle was suspended in a vertical tube through which modified Krebs solution flowed. The modified Krebs solution in one vertical arm was kept at room temperature to keep the muscle inactive and maintain a stable potential against which the test solution in the other vertical arm, kept at 34° C, could be compared. The potential difference between the two sucrose/modified Krebs solution junctions, 6 mm apart, were recorded by means of a pair of $Ag/AgCl_2$ electrodes connected by a 3 M KCl agar-agar bridge to a directly coupled differential amplifier and the signals were displayed on an oscilloscope from which recordings were photographed.

One beta adrenoceptor blocking agent was tested on each taenia coli and at least four experiments were performed for each drug. A constant flow rate was maintained through the vertical arms and a system of three-way taps facilitated a rapid change from control to test solution. The contact time of the agonists was 2 min with a 10 min recovery period between doses. Antagonists acted for 5 min before the block produced was challenged with the agonist.

Experiments were performed using taenia coli from untreated guinea-pigs and guinea-pigs pretreated with reserpine. The reserpine-treated guinea-pigs were injected with reserpine 1 mg/kg intraperitoneally (Crout, Muskus & Trendelenberg, 1962) twice at 24 hr intervals and killed 48 hr after the initial dose. In other experiments the responses of the normal taenia coli to adrenaline were challenged with beta blocking agents in the presence of phentolamine ($1{\cdot}0 \times 10^{-5}$ g/ml.). The effects of the beta blocking agents were observed on the normal or metabolically depressed taenia coli preparations. The normal taenia coli preparations were superfused with modified Krebs solution (Bulbring, 1953 ; NaCl 7·8, KCl 0·35, NaH_2PO_4 0·165,

$NaHCO_3$ 1·37, $CaCl_2$ 0·2, $MgCl_2$ 0·01, glucose 1·40 g/l.) aerated with 95% oxygen and 5% carbon dioxide at pH 7·01. Metabolically depressed taenia coli preparations were produced by superfusing with modified Krebs solution devoid of glucose.

The drugs used were: propranolol (Inderal, I.C.I. Ltd.); pronethalol (Alderlin, I.C.I. Ltd.); INPEA (N-isopropyl-*p*-nitrophenylethanolamine hydrochloride); MJ 1999 (4 (2-isopropylamino-1-hydroxyethyl) methanesulphonanilide hydrochloride) (Mead Johnson Ltd.; MJ 1998 (4 (2-methylamino-l-hydroxypropyl) methanesulphonanilide hydrochloride) (Mead Johnson Ltd.); tyramine hydrochloride; procaine hydrochloride (British Drug Houses Ltd.); dichloroisoprenaline hydrochloride; atropine sulphate (British Drug Houses Ltd.); phentolamine (Rogitine, Ciba Ltd.); reserpine (Serpasil, Ciba Ltd.).

All concentrations quoted refer to the appropriate salt.

Results

Normal taenia coli preparation

On the normal taenia coli preparation, pronethalol ($2{\cdot}5 \times 10^{-8}$ to $1{\cdot}0 \times 10^{-7}$ g/ml.); INPEA ($5{\cdot}0 \times 10^{-8}$ to $2{\cdot}0 \times 10^{-6}$ g/ml.); MJ 1999 ($5{\cdot}0 \times 10^{-8}$ to $1{\cdot}0 \times 10^{-7}$ g/ml.); MJ 1998 ($2{\cdot}5 \times 10^{-7}$ to $7{\cdot}5 \times 10^{-7}$ g/ml.); dichloroisoprenaline ($1{\cdot}0 \times 10^{-8}$ to $1{\cdot}0 \times 10^{-5}$ g/ml.) and tyramine ($2{\cdot}5 \times 10^{-6}$ g/ml.) decreased the electrical excitability of the smooth muscle, increasing the resting membrane potential and abolished spontaneous spike potential activity. In all experiments the spontaneous spike potential activity was abolished before the membrane became hyperpolarized. The hyperpolarization produced by the beta adrenoceptor blocking agents varied from 3 to 16 mV.

In the presence of the alpha adrenoceptor blocking agent phentolamine ($1{\cdot}0 \times 10^{-5}$ g/ml.) the beta blocking agents and tyramine no longer produced a hyperpolarization of the cell membrane, but the suppression of spontaneous spike potential

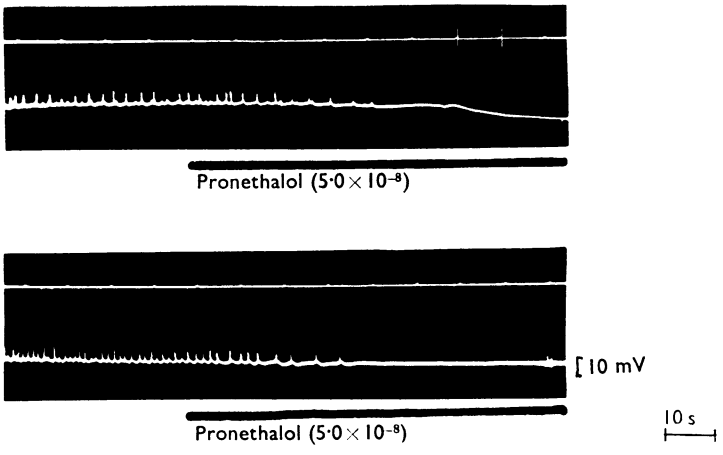

FIG. 1. The upper recording shows the effects of pronethalol ($5{\cdot}0 \times 10^{-8}$ g/ml.) on the taenia coli preparation. Pronethalol produces a cessation of spontaneous spike potential activity, followed by a slowly developing hyperpolarization of the cell membrane. The lower tracing shows the effects of pronethalol ($5{\cdot}0 \times 10^{-8}$ g/ml.) on the taenia coli superfused with modified Krebs solution containing phentolamine ($1{\cdot}0 \times 10^{-5}$ g/ml.). Pronethalol still produces a cessation of spontaneous spike potential activity but no hyperpolarization of the cell membrane. The horizontal bars indicate the onset and duration of drug action. Time marker 10 s.

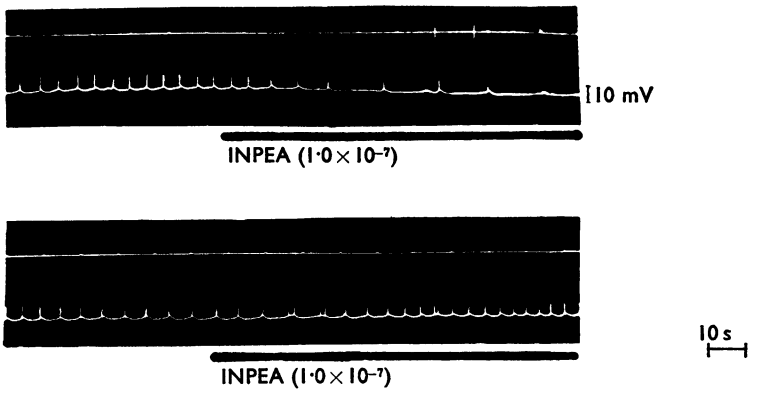

FIG. 2. The upper tracing shows the effects of INPEA ($1{\cdot}0 \times 10^{-7}$ g/ml.) on the taenia coli preparation. INPEA produced a reduction in the spontaneous spike potential activity and a hyperpolarization of the cell membrane. The lower tracing shows the effects of INPEA ($1{\cdot}0 \times 10^{-7}$ g/ml.) on the taenia coli superfused with modified Krebs solution containing phentolamine ($1{\cdot}0 \times 10^{-5}$ g/ml.) and propranolol ($2{\cdot}5 \times 10^{-6}$ g/ml.). INPEA no longer produced a reduction in the spontaneous spike activity nor a hyperpolarization of the cell membrane. The horizontals bars indicate the onset and duration of drug action. Time marker 10 s.

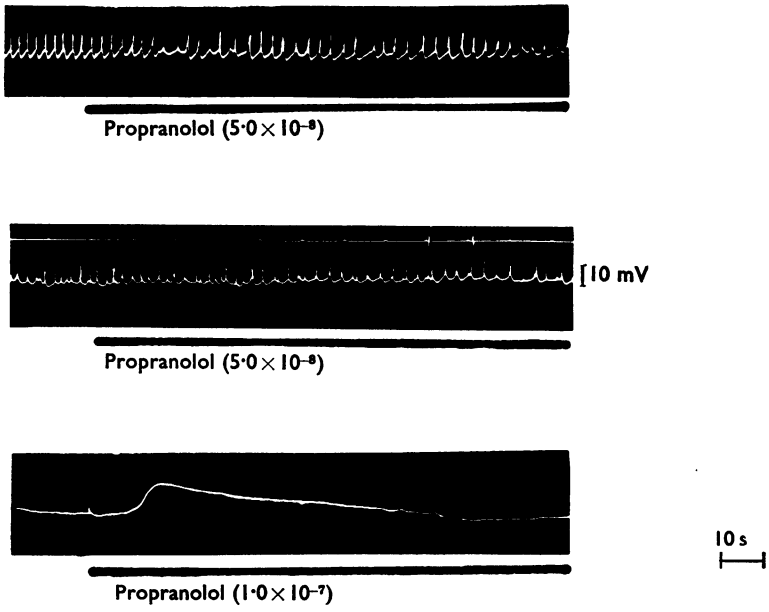

FIG. 3. The upper tracing shows the effects of propranolol ($5{\cdot}0 \times 10^{-8}$ g/ml.) on the taenia coli preparation. Propranolol reduces the frequency of the spontaneous spike potentials, but does not raise the membrane potential. The centre tracing shows the effects of propranolol ($5{\cdot}0 \times 10^{-8}$ g/ml.) on the taenia coli taken from a guinea-pig treated with reserpine (2·0 mg/kg). As in the untreated preparation, propranolol ($5{\cdot}0 \times 10^{-8}$ g/ml.) reduced the frequency of the spontaneous spike potentials, but had no effect on the membrane potential. The lower tracing shows the effect of propranolol ($1{\cdot}0 \times 10^{-7}$ g/ml.) on the metabolically depressed taenia coli preparation. Propranolol now produces a depolarization. The horizontal bars indicate the onset and duration of drug action. Time marker 10 s.

activity was still observed (Fig. 1). Propranolol, in concentrations up to $1{\cdot}0 \times 10^{-5}$ g/ml., had no effect on the loss of spontaneous spike activity or the hyperpolarization of the cell membrane produced by the other beta blocking agents. However, when the taenia coli preparations were superfused with modified Krebs solution containing a mixture of phentolamine ($1{\cdot}0 \times 10^{-5}$ g/ml.) and propranolol ($2{\cdot}5 \times 10^{-6}$ g/ml.), pronethalol, INPEA (Fig. 2), MJ 1999, MJ 1998, dichloroisoprenaline and tyramine no longer produced their sympathomimetic effects.

Propranolol ($2{\cdot}5 \times 10^{-8}$ to $5{\cdot}0 \times 10^{-8}$ g/ml.) reduced the frequency of the spontaneous spike activity but did not completely abolish them and did not produce a hyperpolarization of the cell membrane (Fig. 3).

Propranolol and pronethalol ($7{\cdot}5 \times 10^{-5}$ g/ml.) and procaine ($1{\cdot}5 \times 10^{-4}$ g/ml.) inhibited the spontaneous spike potential activity and lowered the resting membrane potential of the taenia coli. The hyperpolarization produced varied from 9 to 21 mV. These effects were not seen with INPEA, MJ 1999 or MJ 1998 in concentrations of $1{\cdot}5 \times 10^{-4}$ g/ml.

In the presence of phentolamine ($1{\cdot}0 \times 10^{-5}$ g/ml.), propranolol ($2{\cdot}5 \times 10^{-8}$ g/ml.), pronethalol ($1{\cdot}0 \times 10^{-5}$ g/ml.), MJ 1999 ($1{\cdot}0 \times 10^{-5}$ g/ml.), INPEA ($1{\cdot}0 \times 10^{-5}$ g/ml.) and MJ 1998 ($8{\cdot}0 \times 10^{-5}$ g/ml.) prevent the loss of spontaneous spike activity produced by adrenaline $2{\cdot}0 \times 10^{-8}$ g/ml. Antagonism of the adrenaline responses with dichloroisoprenaline could not be shown because dichloroisoprenaline ($1{\cdot}0 \times 10^{-5}$ g/ml.) exhibited sympathomimetic activity.

Metabolically depressed taenia coli preparation

Pronethalol, INPEA, MJ 1999, MJ 1998, dichloroisoprenaline and tyramine in concentrations used to produce sympathomimetic effects in the normal taenia coli preparation and propranolol ($2{\cdot}0 \times 10^{-8}$ g/ml.) (Fig. 3) all produced a depolarization of the cell membrane. The depolarizations produced by the beta blocking agents

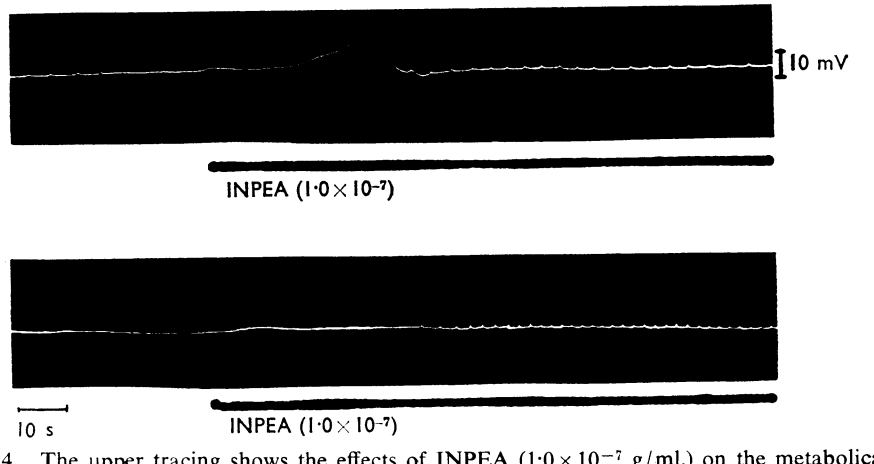

FIG. 4. The upper tracing shows the effects of INPEA ($1{\cdot}0 \times 10^{-7}$ g/ml.) on the metabolically depressed taenia coli preparation. INPEA produced a slowly developing depolarization of the cell membrane, which was followed by increased spontaneous spike potential activity. The lower tracing shows the effects of INPEA ($1{\cdot}0 \times 10^{-7}$ g/ml.) on the metabolically depressed taenia coli preparation, bathed in modified Krebs solution containing phentolamine ($1{\cdot}0 \times 10^{-6}$ g/ml.). Note, in the presence of phentolamine, INPEA no longer produces a depolarization of the cell membrane of the taenia coli, but still initiated spike potential activity. The horizontal bars indicate the onset and duration of drug action. Time marker 10 s.

were of the order of 5 to 28 mV. Following the depolarization, INPEA (Fig. 4), MJ 1999, MJ 1998, dichloroisoprenaline and tyramine always initiated spike potential activity or when they were not totally suppressed (14% of the experiments), increased the frequency of the spontaneous spike potentials. This effect was also produced in one in five experiments with propranolol and three in five experiments with pronethalol. The depolarization of the cell membrane produced by these agents was abolished by phentolamine ($5{\cdot}0 \times 10^{-6}$ g/ml.) but not by atropine ($1{\cdot}0 \times 10^{-5}$ g/ml.). The increased spike potential activity which followed the depolarization was not abolished by either phentolamine or atropine.

Reserpine treated taenia coli preparation

Treatment with reserpine did not modify the effects of the beta blocking agents on the normal or metabolically depressed taenia coli preparation. The hyperpolarizations produced by the beta blocking agents varied from 3 to 10 mV and the depolarizations produced on the metabolically depressed taenia coli from 4 to 20 mV. Sympathomimetic effects were not elicited by $2{\cdot}5 \times 10^{-6}$ g/ml. tyramine, but when the concentration was increased to $1{\cdot}0 \times 10^{-5}$ g/ml. a typical sympathomimetic effect was produced.

Reserpinization affected the size of the spontaneous spike potentials, which were smaller than in control preparations.

Discussion

Pronethalol, INPEA, MJ 1999, MJ 1998 and dichloroisoprenaline abolish the spontaneous spike activity and produce a hyperpolarization of the cell membrane of the smooth muscle of the taenia coli. These effects are similar to those observed for catecholamines (Burnstock, 1958b ; Bulbring, 1960) using the same tissue and technique. These effects of the beta adrenoceptor blocking agents were shown to be mediated through adrenoceptors by superfusing the taenia coli preparation with Krebs solution containing phentolamine and propranolol. In the presence of phentolamine alone, the beta adrenoceptor blocking agents abolish the spontaneous spike activity but do not produce a hyperpolarization of the cell membrane. The presence of propranolol alone did not modify the responses to the other beta blocking agents. In the presence of phentolamine and propranolol, however, pronethalol, INPEA, MJ 1999, MJ 1998 and dichloroisoprenaline had no effect on the spontaneous spike activity or membrane potential of the smooth muscle of the taenia coli. These observations suggest that the hyperpolarization of the cell membrane is mediated through alpha receptors and that inhibition of spontaneous spike potential activity is a beta effect. These findings are in agreement with the observation of Bulbring & Tomita (1968) that the beta effect, the inhibition of spike generation, cannot be antagonized by propranolol if the alpha effect, the hyperpolarization of the cell membrane, persists.

Low concentrations of propranolol reduce the frequency of the spontaneous spike discharges but have no effect on the membrane potential. This reduction in spontaneous spike frequency may be a weak beta receptor stimulant activity.

In high concentrations, propranolol and pronethalol lowered the resting membrane potential and inhibited spontaneous spike potential activity. This effect of propranolol (Morales-Aguilera & Vaughan Williams, 1965) and pronethalol (Gill & Vaughan Williams, 1964) is probably produced by a local anaesthetic action.

In the presence of phentolamine, propranolol, pronethalol, INPEA, MJ 1999 and MJ 1998 in concentrations which are 20–1,000 times greater than those which produce sympathomimetic effects, antagonize the beta receptor stimulant effects of adrenaline.

All the beta adrenoceptor blocking agents produce a slow depolarization of the cell membrane and often initiate spike potential activity when applied to the metabolically depressed taenia coli preparation. The slow depolarization is similar to that produced by noradrenaline on the metabolically depressed taenia coli (Bulbring, 1960) and dissimilar to the rapid depolarization produced by acetylcholine (Burnstock, 1958a). The depolarization of the cell membrane produced by the beta blocking agents was abolished by phentolamine but not by atropine, demonstrating that the depolarization is mediated through alpha receptors. Phentolamine did not antagonize the initiation of spike potential activity, indicating that this effect is not mediated through alpha receptor stimulation.

The beta adrenoceptor blocking agents could produce their sympathomimetic effects either by a direct intrinsic sympathomimetic action or indirectly by the release of catecholamines stored within the taenia coli. Kayaalp & Karim (1966) suggest, from studies of the pressor effects of propranolol in the hind limb of dogs, that propranolol releases catecholamines from storage sites. Hollands & Vanov (1965), using fluorometric techniques, showed that of all the intestinal tissues the taenia coli is richest in catecholamine content. To ascertain whether the beta blocking agents produce their effects by a direct or indirect action, the catecholamine stores within the taenia coli were depleted with reserpine. The dose of reserpine used was found by Crout *et al.* (1962) to deplete the catecholamine stores in the guinea-pig atria by 98%. Such large depletions are necessary because Crout *et al.* (1962) found that the indirectly elicited tyramine response was not greatly reduced unless the catecholamine content of the tissues was reduced by more than 95%. Although the indirectly elicited tyramine response was not abolished, a greater concentration had to be used to elicit a response in the reserpine-treated preparation than in the normal preparation. The effective concentrations of the beta blocking agents were unaffected by the depletion of the catecholamine stores within the taenia coli. These observations suggest that the beta blocking agents do not produce their sympathomimetic effects in a similar manner to tyramine, but by a direct action on the taenia coli.

Comparison of the sympathomimetic activities of the beta blocking agents on cardiac and smooth muscle show that the agents which are reported to exhibit sympathomimetic activity on cardiac muscle, pronethalol (Somani *et al.*, 1963), INPEA (Meester *et al.*, 1965), MJ 1998 (Stanton *et al.*, 1965) and dichloroisoprenaline (Fleming & Hawkins, 1960 ; Moran & Perkins, 1958) also exhibit sympathomimetic activity on the smooth muscle of the taenia coli. MJ 1999, which is reported to be devoid of sympathomimetic activity on cardiac muscle (Stanton *et al.*, 1965), exhibited sympathomimetic activity on smooth muscle. Propranolol, which is also reported to be devoid of sympathomimetic activity on cardiac muscle (Shanks, 1966), was shown to exhibit sympathomimetic alpha actions on the metabolically depressed taenia coli and, possibly, weak beta actions on the normal taenia coli preparations.

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