Biphasic Effect of 1, 1-dimethyl-4-phenylpiperazinium Iodide (DMPP) on the Guinea-pig Distal Colon

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(Received November 6, 1972)

The ganglion stimulating action of DMPP had been reported in taenia coli smooth muscle but the responses by DMPP were not constant (Weis, 1962; Burnstock et al, 1966; Akubue, 1966; Hobbiger et al, 1969; Bucknell et al, 1964). Burnstock et al (1966) suggested that DMPP caused relaxation of the taenia by stimulation of inhibitory neurones in the enteric plexus. In the present experiment, the effect of DMPP on the contractile activity of the guinea-pig distal colon was investigated, since it is thought that the innervation to the smooth muscle of the guinea-pig distal colon is different functionally from that of the guinea-pig taenia coli. That is, the smooth muscle of the guinea-pig distal colon is considered to relate dominantly with the excitatory neurones in the enteric plexus. The details were discussed in the previous paper (Ohkawa, 1972).

METHODS

Guinea-pigs of either sex, weighing between 300–500g, were used. The animals were stunned with a blow and bled out. A suitable length, 2–2.5cm, of the distal colon was removed. Segments were suspended in an organ bath containing 300ml of the modified Krebs solution bubbled with 95% O_2 and 5% CO_2. The temperature was maintained at between 36 and 37°C with a heated water-bath. The free end of the preparation was connected to a mechano-electronic transducer. The tension was recorded by a conventional apparatus. The composition of the modified Krebs solution was as follows (mM); NaCl, 120.7; KCl, 5.9; $CaCl_2$, 2.5; $MgCl_2$, 1.2; $NaHCO_3$, 15.5; NaH_2PO_4, 1.2 and glucose 11.5. The following drugs were used; 1,1-dimethyl-4-phenylpiperazinium iodide (DMPP), hexamethonium bromide, atropine sulfate, bevonium methylsulfate, hyoscine-N-butylbromide, physostigmine sulphate, guanethidine sulfate, pentolamine methansulfonate, phenoxybenzamine hydrochloride (dibenzyline), hyoscine hydrobromide and tetrodotoxin. The concentrations of the used drugs were given in g/ml.

RESULTS

All preparations showed the spontaneous mechanical activity in normal solution. The magnitude and interval in the successive phasic contraction were irregular. Low concentrations ($5 \times 10^{-8} - 5 \times 10^{-7}$ g/ml) of DMPP exhibited a slight excitatory effect on the tone, i.e., the tone was gradually increased during exposure to DMPP. When high concentrations ($5 \times 10^{-6} - 5 \times 10^{-5}$ g/ml) was applied, a contraction followed by a relaxation was observed in all preparations. The smooth recovery was obtained after 15 min exposure to DMPP 5×10^{-6} g/ml solution while it took long period to recover to the normal activity in DMPP 5×10^{-5} g/ml. The concentration of DMPP 5×10^{-6} g/ml, therefore, was used in the following experiments. Fig. 1 shows the effects of DMPP in various concentrations on the contractile activity of distal colon.

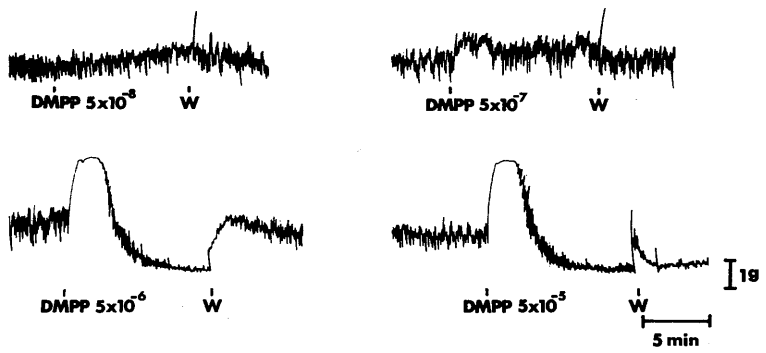

Fig. 1· Effects of DMPP in various concentrations on the contractile activity of guinea-pig distal colon. The concentrations of DMPP were given in g/ml. W; Washing out with normal solution. Biphasic response was observed in high concentrations of DMPP.

Fig. 2. Effect of DMPP in tetrodotoxin on the contractile activity of guinea-pig distal colon.
Biphasic response (control) was observed by DMPP 5×10^{-6} g/ml. When applied TTX 10^{-7} g/ml, the tone was reduced and the magnitude of phasic contraction was also reduced. DMPP 5×10^{-6} g/ml was applied during TTX. No response was observed by additional DMPP.

When tetrodotoxin 10^{-7}g/ml was applied, the tone and the magnitude of phasic contraction were reduced, i. e., tetrodotoxin itself showed the relaxant effect on the contractile activity. After 15 min exposure to tetrodotoxin, DMPP 5×10^{-6} g/ml had nearly no effect on the contractile activity. Fig. 2 shows the effect of DMPP in tetrodotoxin solution on the contractile activity of distal colon. Hexamethonium, ganglion blocking drug, also showed a relaxant effect on the tone. The concentrations of hexamethonium from 10^{-8} to 10^{-5}g/ml were tested. The similar tendency was observed in above concentrations of hexamethonium. The additional application of tetrodotoxin 10^{-7}g/ml during exposure to hexamethonium 10^{-6}g/ml or 10^{-5}g/ml produced a further relaxation. In contrast, the additional application of hexamethonium 10^{-5}g/ml during exposure to tetrodotoxin 10^{-7}g/ml did not show the relaxant effect.

When physostigmine $10^{-8}-2\times10^{-8}$g/ml was applied, the increase in the tone was observed. The contracture was produced by the application of physostigmine 10^{-7}g/ml. It recovered slowly to normal tone after washing by normal solution. After the increase in tone was generated by physostigmine 2×10^{-8}g/ml, the additional tetrodotoxin 10^{-7}g/ml exhibited the relaxant effect. After 15 min exposure to tetrodotoxin 10^{-7}g/ml, the additional physosigmine 2×10^{-8} g/ml produced the slight increase in the tone. The increase by physostigmine in tetrodotoxin may due to a direct action to smooth muscle. DMPP5×10^{-6}g/ml applied in physostigmine 2×10^{-8}g/ml produced the biphasic response but the followed relaxation was diminished in a short time. That is, the tone was again increased to the initial level during exposure to DMPP. Further addition of tetrodotoxin 10^{-7}g/ml was showed the relaxant action. Fig. 3 shows the effect of DMPP in physostigmine.

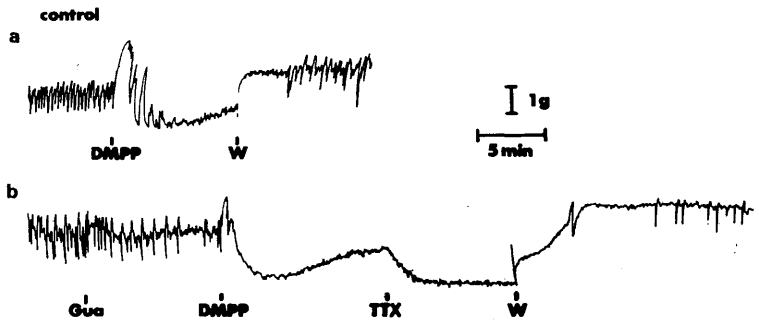

Fig. 3. Effect of DMPP in physostigmine on the contractile activity of guinea-pig distal colon.
a, control; Biphasic response was produced by DMPP 5×10^{-6} g/ml. b, In physostigmine 2×10^{-8} g/ml, the tone was increased. DMPP 5×10^{-6} g/ml during physostigmine produced the biphasic response but the relaxation was diminished. The tone again increased to the initial level. TTX 10^{-6} g/ml reduced the tone.

All cholinergic blocking drugs, atropine, hyoscine-N and bevonium, the concentration of these drugs were 10^{-5}g/ml, had a relaxant action on the contractile activity. The tone and the magnitude of the phasic contraction were reduced. After 15 min exposure to atropine 10^{-5}g/ml, DMPP 5×10^{-6}g/ml was applied in atropine. The biphasic response was observed but the magnitude of the initial contraction was reduced considerably. Further additional tetrodotoxin after 15 min in DMPP with atropine produced a slight decrease in tone.

Furness (1969) and Bennett (1969) reported that hyoscine blocked the excitatory junction potential in smooth muscle. In the present experiment, to find out the effect of DMPP on the excitatory neurones, hyoscine was also examined. Hyoscine 10^{-5}g/ml produced the decrease in tone but the phasic contraction was not abolished. DMPP 5×10^{-5}g/ml was applied after exposure to hyoscine 10^{-5}g/ml. DMPP still produced the biphasic response in hyoscine. The duration of the initial contraction by DMPP was slightly reduced but not altered in magnitude. Fig. 4 shows the effect of DMPP in the hyoscine solution on the contractile activity of distal colon.

Adrenergic blocking drugs, guanethidine, pentolamine and dibenzyline were used. All these drugs at the concentration of 10^{-5}g/ml exhibited the small inhibitory effect on the contractile activity of distal colon. During exposure to guanethidine 10^{-5}g/ml, DMPP 5×10^{-5}g/ml produced the biphasic response but the response was similar to that of control. The effect of DMPP in guanethidine were shown in Fig. 5.

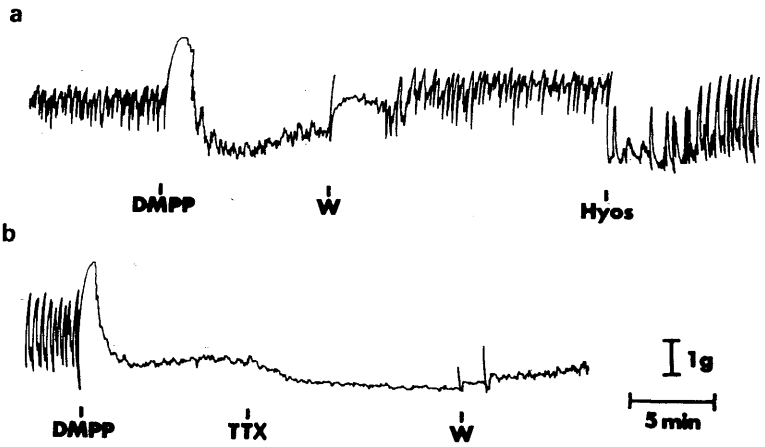

Fig. 4. Effect of DMPP in hyoscine on the contractile activity of guinea-pig distal colon.
a, control; DMPP 5×10^{-6} g/ml produced the biphasic response. Hyoscine 10^{-5} g/ml exhibited the relaxant effect but the phasic contraction was not abolished. b (continuous record from a), DMPP 5×10^{-6} g/ml in hyoscine 10^{-5} g/ml produced the biphasic response but the initial contraction was decreased. TTX, 10^{-6} g/ml.

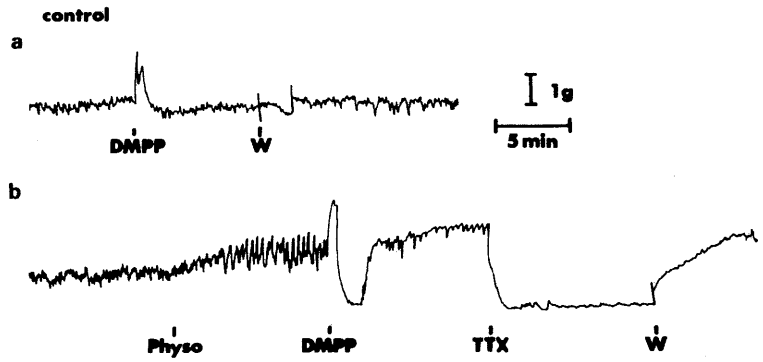

Fig. 5. Effect of DMPP in guanethidine on the contractile activity of guinea-pig distal colon.
a, control; Biphasic response was observed by DMPP 5×10^{-6} g/ml. b, Guanethidine 10^{-5} g/ml showed slight changes in the activity. DMPP 5×10^{-6} g/ml during guanethidine produced the biphasic response. TTX' 10^{-6} g/ml.

DISCUSSION

The effects of the ganglion stimulant DMPP on the isolated taenia of the guinea-pig vary with the tone of the preparation; contractions, relaxations or complex responses have been obtained with DMPP (Weis, 1962; Burnstock et al, 1966; Akubue, 1966; Hobbiger et al, 1969). Winbury (1959) suggested that a direct relaxant effect of DMPP on the vascular smooth muscle of the dog hind limb. In the smooth muscle used in the present experiment, biphasic response was obtained by DMPP. The relaxing effect of DMPP could be due to an action to the smooth muscle itself. However this possibility can probably be disregarded, since both the contraction and relaxation on the isolated distal colon in response to DMPP can be abolished by tetrodotoxin.

In high concentration of DMPP, the contraction followed by the relaxation was observed. The biphasic response may due to stimulating action of DMPP on the neurones in enteric plexus of the gut. In the previous paper (Ohkawa, 1972), the possibility that the excitatory neurones may be dominant in the enteric plexus of the guinea-pig distal colon was discussed. In fact, the relaxation produced by tetrodotoxin and the inhibitory effect by hexamethonium were also observed in the present experiment. The order of the contraction and relaxation might be depend on the sensitivity of the excitatory and inhibitory neurones to DMPP, i.e., the low concentration of DMPP may stimulate the excitatory neurones, since only excitatory effect was observed in low concentrations of DMPP. High concentration of DMPP may be necessary to stimulate the inhibitory neurones. The "rebound excitation" (Hobbiger et al, 1969) was not observed in the present experiment.

Hobbiger et al (1969) concluded that contractions of the taenia produced by DMPP were due to an action of the drug on intramural cholinergic ganglia. This conclusion agrees with that of Weis (1962) and Akubue (1966). Results obtained in the present experiment also suggest that DMPP stimulates the cholinergic neurones in the enteric plexus and produces the initial contraction. The evidences that initial contraction by DMPP was suppressed in atropine and the relaxation by DMPP during physostigmine was again increased to the normal tone may support above conclusion. Bucknell et al (1964) also reported that physostigmine abolished the relaxation of the isolated taenia coli produced by DMPP.

The blocking action of hyoscine to the excitatory junction potential had been reported (Furness, 1969 ; Bennett, 1969). In the plexus-free smooth muscle, the spontaneous excitatory junction potential was not observed (Bennett, 1969). The burst activity of the enteric neurones of cat intestine also reported (Ohkawa et al, 1971). It is speculated from above evidences that 1) the burst activity of some enteric neurones has an excitatory effect to smooth muscle, 2) it may be block by hyoscine and 3) the DMPP effect is decreased by hyoscine. In fact, the initial contraction was reduced by hyoscine. This result suggests that DMPP acts to the excitatory neurones in enteric plexus. Hobbiger et al (1969) had obtained similar results on the taenia of guinea-pig caecum. However the possibility that DMPP has a stimulating action to the non-cholinergic excitatory neurones cannot be excluded.

It reported that DMPP caused a relaxation of the taenia coli (Bucknell et al, 1964; Burnstock et al, 1966; Weis, 1962). Burnstock et al (1966) suggested that DMPP stimulated the inhibitory neurones in enteric plexus of the taenia coli preparation. The relaxation may produce by the ganglion stimulating action of DMPP since DMPP showed no direct action to smooth muscle and the presence of the inhibitory neurones in the guinea-pig distal colon was known (Holman et al, 1965 ; Furness, 1969).

The response to DMPP was blocked or decreased by pronethalol or yohimbine (Bucknell et al, 1964 ; Weis, 1962). Guanethidine also decreased the response by DMPP (Burnstock et al, 1969). However, in the present experiment, guanethidine did not decreased the response to DMPP. This result suggests that DMPP stimulates the non-adrenergic inhibitory neurones and the adrenergic inhibitory neurones in the distal colon are minor functionally. The difference on the responses of taenia coli and distal colon to DMPP in guanethidine may due to the difference of functional distribution of the adrenergic and non-adrenergic inhibitory neurones in enteric plexus.

SUMMARY

1. Effects of DMPP on the contractile activity of guinea-pig distal colon were

investigated in normal solution and in various autonomic blocking drugs.

2. Biphasic response was produced by DMPP and the effect of DMPP was blocked by tetrodotoxin. The initial contraction in response to DMPP was decreased by atropine and hyoscine. The relaxation in response to DMPP was diminished in physostigmine.

3. The effect of DMPP in guanethidine was nearly similar to that in control.

4. It is concluded that DMPP stimulated the cholinergic excitatory neurones in enteric plexus and produced the initial contraction. The relaxation by DMPP may due to the stimulating action of DMPP on non-adrenergic inhibitory neurones.

REFERENCES

1) Akubue, P.I.: The site of action of drugs on the isolated taenia caeci from the guinea-pig. *Br. J. Phaemac. Chemother.*, **27**: 347–365, 1966.
2) Ambache, N. & Freeman, M. A.: Atropine-resistance longitudinal muscle spasms due to excitation of non-cholinergic neurones in Auerbach's plexus. *J. Physiol.*, **199**: 705–727, 1968.
3) Bell, C.: Differential effects of tetrodotoxin on sympathomimetic actions of nicotine and tyramine. *Br. J. Pharmac. Chemother.*, **32**: 96–103, 1968.
4) Bennett, T.: Nerve-mediated excitation and inhibition of the smooth muscle cells of the avian gizzard. *J. Physiol.*, **204**: 669–686, 1969.
5) Bucknell, A. & Whitney, B.: A preliminary investigation of the pharmacology of the human isolated taenia coli preparation. *Brit. J. Pharmacol.*, **23**: 164–175, 1964.
6) Burnstock, G., Campbell, G. & Rand, M. J.: Inhibitory innervation of the taenia of the guinea-pig caecum. *J. Physiol.*, **182**: 504–526, 1966.
7) Burnstock, G., Campbell, G., Satchell, D. & Smythe, A.: Evidence that adenosine triphosphate or a related nucleotides is the transmitter substance released by non-adrenergic inhibitory nerves in the gut. *Br. J. Pharmacol.*, **40**:668–688, 1970.
8) Evans, D.H.L. & Schield, H.O.: The reactions of plexus-free circular muscle of cat jejunum to drugs. *J. Physiol.*, **119**: 376–399, 1953.
9) Furness, J. B.: An electrophysiological study of the innervation of the smooth muscle of the colon. *J. Physiol.*, **205**: 549–562, 1969.
10) Hobbiger, F., Mitchelson, F. & Rand, M. J.: The actions of some cholinomimetic drugs on the isolated taenia of the guinea-pig caecum. *Br. J. Pharmac.*, **36**: 53–69, 1969.
11) Holman, M. E. & Hughes, J. R.: Inhibition of intestinal smooth muscle. *Aust. J. exp. Biol. Med. Sci.*, **43**: 277–290, 1968.
12) Ohkawa, H.: Inhibitory effect of tetrodotoxin on the contractile activity on guinea-pig ileum and distal colon. *Br. J. Pharmacol.*, (in submit) 1972.
13) Weis, J.: Biphasic response of guinea-pig's taenia coli induced by 1,1-dimethyl4-phenylpiperazinium (DMPP). *Acta Pharmacol. Toxicol.*, **19**: 121–128, 1962.
14) Winbury, M. M.: Mechanism of the local vascular actions of 1,1-dimethyl-4-phenylpiper azinium. *J. Physiol.*, **147**: 1–13, 1959.