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EFFECTS OF DOPAMINE ON ADRENERGIC NEUROMUSCULAR TRANSMISSION IN THE GUINEA-PIG VAS DEFERENS

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1 In the isolated vas deferens of the guinea-pig, contractile responses to adrenergic nerve stimulation at 2 Hz were depressed by exogenous dopamine (5 μM) and this effect was abolished in the presence of phentolamine (0.3 μM), suggesting that it was due to an agonist action of dopamine on α-adrenotors.

2 The depression by dopamine (5 μM) of contractile responses to nerve stimulation was correlated with reduction in amplitude of single excitatory junction potentials (e.j.ps) evoked by nerve stimulation, but not with depression of spontaneous junction potentials.

3 By contrast, during repetitive nerve stimulation at 1 Hz the depressant effect of dopamine on e.j.p. amplitude became less pronounced, due to the amount of facilitation being greater than that occurring under control conditions in the same cell.

4 The α-adrenoceptor antagonist, phentolamine (10 μM), also increased the amount of facilitation during repetitive nerve stimulation.

5 In the presence of phentolamine (10 μM), the depressant effect of dopamine (5 μM) on single e.j.ps was abolished but its enhancing effect on facilitation was not reduced.

6 It is suggested that the enhancement of facilitation during repetitive stimulation by both dopamine and phentolamine is independent of their actions on presynaptic α-adrenoceptors.

Introduction

Release of noradrenaline from adrenergic axons in response to nerve stimulation is known to be enhanced by α-adrenoceptor antagonists and to be depressed by α-adrenoceptor agonists (Farnebo & Hamberger, 1971; Kirpekar & Puig, 1971; Starke, 1971; 1972; Enero, Langer, Rothlin & Stefano, 1972). Such results have led to the suggestion that presynaptically located α-adrenoceptors may be involved in the feedback modulation of adrenergic neuromuscular transmission (see Langer, 1974; Starke, 1977; Westfall, 1977 for recent reviews).

In the guinea-pig vas deferens, contractile responses to low-frequency adrenergic nerve stimulation are depressed by exogenous dopamine, and this effect is prevented by phentolamine, suggesting that it is mediated through an agonist action of dopamine on α-adrenoceptors (Bell & Matalanis, 1977). The purpose of the present paper is to examine the effects of dopamine on the process of adrenergic neuromuscular transmission in this tissue.

Some of these results were described at a meeting of the Physiological Society (Bell, 1978).

Methods

Vasa deferentia were removed from adult (400 to 700 g) guinea-pigs killed by cervical dislocation, and bathed in modified Krebs solution (Hukovic, 1961) containing 100 μg/ml ascorbic acid, at 36°C.

Mechanical responses to intramural nerve stimulation were recorded as described previously (Bell & Matalanis, 1977).

For investigation of electrical events during neuromuscular transmission, the vasa were bathed in Krebs solution flowing at a rate of 2 ml/min. Glass microelectrodes filled with 2 M KCl were used to record trans-membrane potentials from within superficially located smooth muscle cells. The microelectrodes had resistances of 80 to 200 MΩ and the criteria for intracellular recording were a stable resting membrane potential of at least 60 mV and the presence of spontaneous excitatory junction potentials at least 5 mV in amplitude. The postganglionic branches of the hypogastric nerve were stimulated close to their entry into the vas deferens with Pt wire electrodes which delivered short trains (12 to 20 impulses) of 0.1 ms square wave pulses at 0.4 to 1 Hz from a Grass S44 stimu-

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lator. In order to avoid significant errors due to non-linear summation (Martin, 1955), the stimulus voltage was adjusted so that facilitated excitatory junction potentials were less than 10 mV in amplitude. Successive stimulating trains were separated by at least 60 s to allow decay of facilitation. Dopamine hydrochloride and phentolamine mesylate (Regitine, Ciba-Geigy) were applied to the tissue by changing the inflow to the tissue bath from normal Krebs solution to Krebs containing the appropriate drug, without alteration of flow rate. The time taken for 90% replacement of the contents of the bath was approximately 10 min (as estimated from determinations of changes in bath Cl^- concentration during periods of perfusion with solutions of known Cl^- concentration). For each cell studied, data were first recorded under control conditions and then the drug-containing solution was allowed to enter the bath. After a time interval of 10 min or more, further data were recorded. In some cells, the reversibility of drug-induced changes was determined by recording a third set of data after the drug had been washed out of the bath.

Statistical significance of differences between means was assessed by a two-tailed Students' *t* test for paired data.

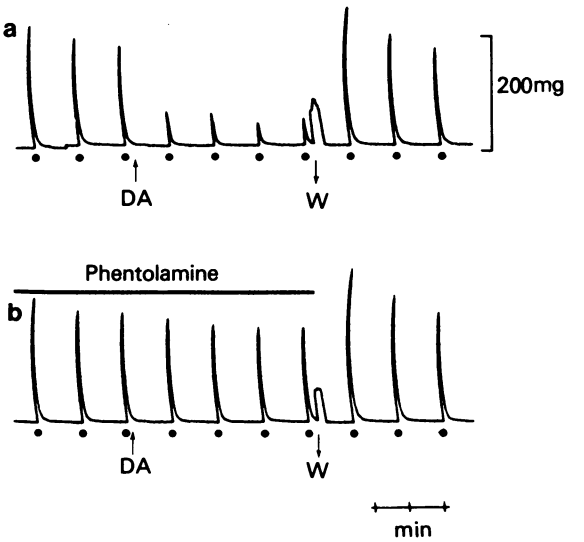

Figure 1 Twitch responses of guinea-pig isolated vas deferens to postganglionic nerve stimulation (0.5 ms, 2 Hz for 5 s every 80 s) at black dots. (a) Dopamine (DA, 5 μM) added to the bath depressed the responses and this effect was reversed after washing (W). (b) In the presence of phentolamine (0.3 μM) the depressant effect of dopamine was abolished. Time marker: 1 min intervals.

Results

Effects of dopamine on mechanical responses to nerve stimulation

Twitch responses of the vas deferens to postganglionic nerve stimulation (0.5 ms pulses, 2 Hz for 5 s every 80 s) were reduced in amplitude in the presence of 1 to 5 μM dopamine. The extent of the dopamine-induced depression of responses was concentration-dependent and at a concentration of 5 μM was between 40% and 70% in different preparations. As reported previously (Bell & Matalanis, 1977) concentrations of dopamine higher than this caused contraction of the vas deferens, which prevented assessment of amplitude of neurogenic responses. The 5 μM concentration was therefore chosen for further study as the concentration producing maximum effect. The depressant effect of dopamine was quickly reversed on washing the organ bath free of the drug and could be repeated several times in any one preparation. Dopamine had no depressant effect when added in the presence of 0.3 μM phentolamine (Figure 1).

Effects of dopamine on neuromuscular transmission

Dopamine on spontaneous junction potentials At a concentration of 5 μM, dopamine had no discernible effect on the resting membrane potential of any cell studied.

The pattern of spontaneous junction potential (s.j.p.) discharge was examined in 10 cells from three preparations under control conditions and in the presence of 5 μM dopamine. Those s.j.ps with amplitudes less than about 0.6 mV were difficult to distinguish reliably from baseline noise fluctuations: only s.j.ps with amplitudes of at least 1 mV were therefore counted. Although the frequency of the small s.j.ps was slightly lower in the presence of dopamine than under control conditions, no obvious difference in the overall pattern of discharge could be seen. For all cells studied, the mean (±s.e. mean) frequency of s.j.ps was: control 0.29 ± 0.05/s, dopamine 0.27 ± 0.04/s, while the amplitudes of the largest s.j.ps recorded were control 5.9 ± 0.58 mV, dopamine 7.1 ± 0.80 mV. S.j.p. discharges recorded in a typical cell are illustrated in Figure 2.

Dopamine on excitatory junction potentials In the presence of 5 μM dopamine the excitatory junction potential (e.j.p.) evoked by a single impulse was considerably reduced in amplitude (Figure 2). The mean amplitudes recorded in 18 cells from eight prep-

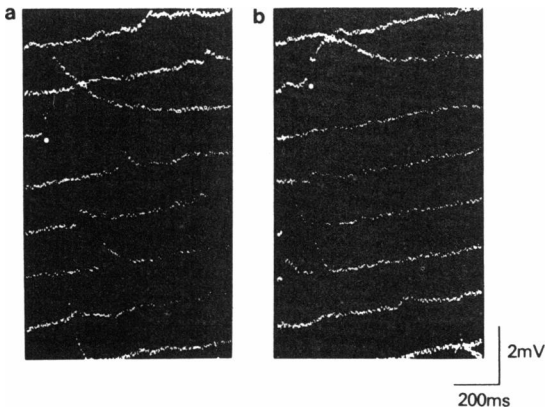

Figure 2 Intracellular recording of membrane potential from a smooth muscle cell in the vas deferens (a) under control conditions and (b) in the presence of 5 μM dopamine. Note the reduction by dopamine of amplitude of the evoked excitatory junction potential (at white dots). Calibrations: 2 mV and 200 ms.

arations were: control 1.66 ± 0.15 mV, dopamine 0.98 ± 0.06 mV. When the percentage reduction for each cell was considered, the mean reduction produced by dopamine was 39 ± 5% ($P < 0.001$).

During repetitive nerve stimulation of the postganglionic nerves to the vas deferens, successive e.j.ps facilitate over the first 6 to 12 impulses of a train, due to increased liberation of transmitter with successive impulses. The number of impulses needed to produce a fully-facilitated e.j.p. is directly related to the stimulation frequency (Burnstock, Holman & Kuriyama, 1964). The effect of dopamine on e.j.p. amplitude was examined during repetitive stimulation at 0.4 Hz in five cells and at 1.0 Hz in 13 cells from five preparations. At each frequency, the degree of depression of the e.j.p. by dopamine became less pronounced with repetitive stimulation. Thus, while the percentage reduction in amplitude of the first e.j.p. of a train was 41 ± 5% for those cells in which stimulation at 0.4 Hz was studied and 37 ± 4% for those in which stimulation at 1.0 Hz was studied, the equivalent reductions of fully facilitated e.j.ps were, respectively, 26 ± 5% and 18 ± 4%. Records taken from a typical cell during stimulation at 1.0 Hz are shown in Figure 3 and pooled data for all the cells studied using this frequency are plotted in Figure 4.

In order to quantitate the effect of dopamine further, the amount of facilitation produced by each successive stimulating impulse was expressed in the form V_n–Vo/Vo, where Vo = the amplitude of the first e.j.p. of the train and V_n = the amplitude of the nth e.j.p. (Mallart & Martin, 1967), and plotted against n. This demonstrated that, at either stimulation frequency, the absolute amount of facilitation which occurred in the presence of dopamine was considerably greater than that which occurred under control conditions (Figure 5). The difference was most prominent after the first 4 to 6 impulses of a train, when

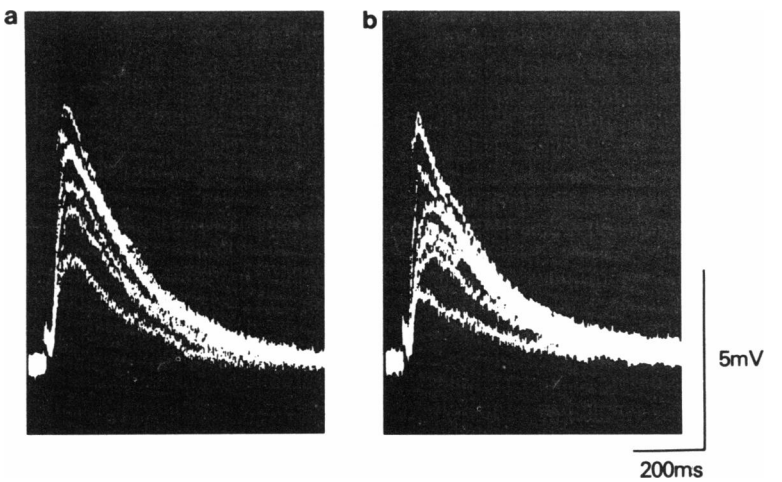

Figure 3 Superimposed excitatory junctional potentials (e.j.ps) evoked by a train of stimuli at 1 Hz (a) under control conditions and (b) in the presence of 5 μM dopamine. Note the depression of the first (smallest) e.j.p. but only minimal depression of the fully-facilitated e.j.p. Calibrations: 5 mV and 200 ms.

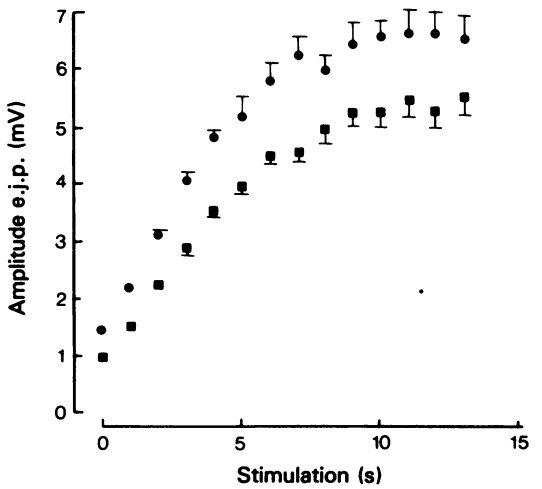

Figure 4 Mean changes in amplitude (mV) of successive excitatory junctional potentials (e.j.ps) during a train of 14 stimuli at 1 Hz, under control conditions (●) and in the presence of 5 μM dopamine (■), for a total of 13 cells. Vertical bars show s.e. mean. Note the progressive attenuation during the train of the depressant effect of dopamine, from 37% reduction in amplitude of the first e.j.p. to 18% reduction of the fully-facilitated e.j.ps.

facilitation began to decline under control conditions but continued to occur linearly in the presence of dopamine for several more impulses.

Both the depressant effect of dopamine on the amplitude of a single e.j.p. and its enhancement of facilitation during repetitive stimulation were reversed after washing the bath free of dopamine, and after recovery could be repeated in the same cell.

The extent to which dopamine affected the latency and rise-time of the fully facilitated e.j.p. was examined in cells from three preparations. No effect on either parameter could be detected (latency: control 17.3 ± 0.65 ms, dopamine 17.0 ± 0.78 ms, $n = 10$; half rise-time: control 16.8 ± 0.34 ms, dopamine 16.6 ± 0.90 ms, $n = 5$).

In a further four cells, the number of axons or axon groups contributing to the fully facilitated e.j.p. was assessed by determining the number of incremental reductions in e.j.p. amplitude during gradual reduction in stimulus voltage (Bell, 1969; Hirst, 1977). In each of the cells examined, between five and seven amplitude plateaux were seen, and in none of the cells was the depression of e.j.p. amplitude by dopamine associated with any change in the number of plateaux.

Interaction of dopamine and phentolamine on excitatory junctional potentials The effect of dopamine on facilitation of e.j.ps during repetitive stimulation was reminiscent of the enhancement of facilitation in the presence of α-adrenoceptor antagonists described for the mouse vas deferens by Bennett & Middleton (1975). It was therefore decided to examine the interaction of effects of dopamine with those of the α-adrenoceptor antagonist phentolamine.

At a concentration of 10 μM, phentolamine had no

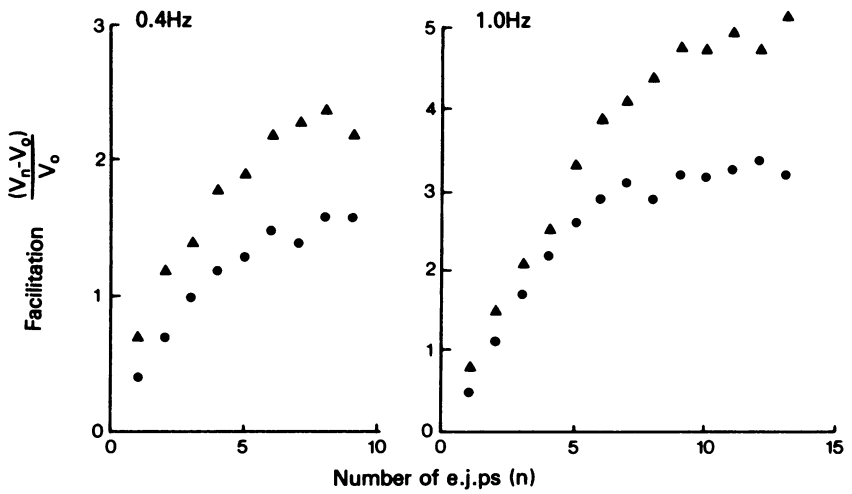

Figure 5 Mean facilitation of successive excitatory junctional potentials (e.j.ps) during repetitive stimulation at (a) 0.4 Hz and (b) 1.0 Hz under control conditions (●) and in the presence of 5 μM dopamine (▲). Pooled data for (a) 5 cells and (b) 13 cells. For each point shown one s.e. mean. was less than 10% of the value of the mean.

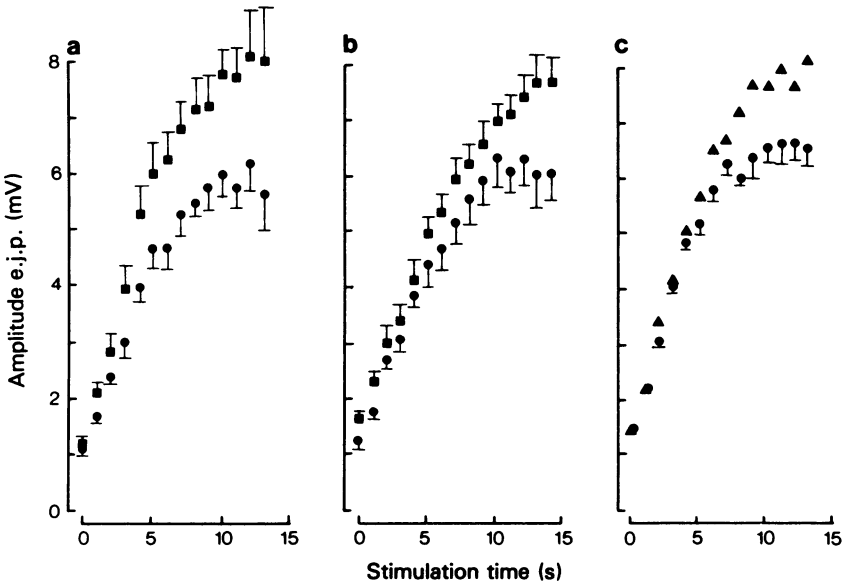

Figure 6 Mean changes in the amplitude (mV) of successive excitatory junctional potentials (e.j.ps) during repetitive stimulation at 1.0 Hz. (a) Under control conditions (●) and in the presence of 10 μM phentolamine (■). (b) In the presence of 10 μM phentolamine (●) and after addition of 5 μM dopamine (■). (c) For comparison, the data from Figure 4 for e.j.p. amplitude under control conditions (●) and in the presence of 5 μM dopamine (▲), replotted so as to discount the depressant effect of dopamine. Vertical bars show s.e. mean

discernible effect on resting membrane potential of any cell studied.

The effect of 10 μM phentolamine on e.j.p. amplitude was examined in nine cells from four preparations. Phentolamine had no effect on the amplitude of the e.j.p. evoked by a single impulse (control 1.1 ± 0.12 mV; phentolamine 1.2 ± 0.17 mV). However, during repetitive stimulation the amount of facilitation which occurred was increased considerably in the presence of phentolamine (Figure 6a), with the result that the amplitude of the fully facilitated e.j.p. was greater ($P < 0.001$). The mean amplitudes recorded were: control 6.2 ± 0.55 mV, phentolamine 8.2 ± 0.76 mV. This effect was reversed following washing the bath free of phentolamine.

In nine cells from three other preparations, the effects of 5 μM dopamine on e.j.p. amplitude were examined in the presence of 10 μM phentolamine. The stimulus voltage was adjusted so that the amplitude of the fully facilitated e.j.p. was similar to that seen under control conditions in the previous series of experiments. Under these circumstances, the absolute amount of facilitation in the presence of phentolamine alone was rather less than was seen in the earlier experiments (Figure 6b cf 6a). While this might have been related to variation between animals, it seems likely to have been at least in part related to the fact that phentolamine was present throughout the experiment, rather than being applied to the tissues only for a few minutes.

When phentolamine was present, the depressant effect of dopamine on amplitude of a single e.j.p. was converted to a slight enhancement, although this was not significant when the pooled data for all cells were analyzed (phentolamine 1.3 ± 0.13 mV, phentolamine plus dopamine 1.7 ± 0.16 mV, $0.2 > P > 0.1$). During repetitive stimulation at 1 Hz, the initial rate of facilitation was unchanged by dopamine, but the process continued over a greater number of impulses than in the presence of phentolamine alone, with the result that the fully-facilitated e.j.p. was significantly ($P < 0.001$) larger than before addition of dopamine (Figure 6b). The mean amplitudes recorded for the fully-facilitated e.j.ps were: phentolamine 6.3 ± 0.49 mV, phentolamine plus dopamine 7.7 ± 0.42 mV.

This result suggested that dopamine might have two independent actions; one to depress e.j.p. amplitude *per se* and the second to enhance the progressive facilitation of successive e.j.ps which occurs during repetitive stimulation. In an attempt to clarify the

situation, the data obtained with dopamine alone were re-assessed. Assuming that the depressant effect of dopamine is constant for all e.j.ps of a repetitive series, then the effect of dopamine on facilitation alone can be derived by multiplying the absolute amplitudes of each control e.j.p. in the train (Figure 4) by the amount of facilitation observed for the same e.j.p. during exposure to dopamine (Figure 5b). The results of this derivation are plotted in Figure 6c. It can be seen that the calculated data resemble very closely the experimental data obtained when dopamine was applied in the presence of phentolamine (Figure 6b).

Discussion

Exogenous dopamine in concentrations of 1 to 5 μM profoundly depresses the amplitude of mechanical responses of the guinea-pig vas deferens to adrenergic nerve stimulation. This effect is not associated with depression of contractile responses to applied noradrenaline and is antagonized by low concentrations of phentolamine but not by the dopamine-receptor antagonist, haloperidol, suggesting the involvement of an α-adrenoceptor on the adrenergic axons (Bell & Matalanis, 1977).

In the present study, the depression of the mechanical response by dopamine has been shown to be correlated with reduction in amplitude of the e.j.p. evoked by a single nerve impulse. As there was no parallel reduction in amplitude of the largest s.j.ps recorded in any of the cells studied or any change in s.j.p. frequency, this effect is unlikely to have been due to an action of dopamine at postsynaptic α-adrenoceptors. Furthermore, the absence of any changes in latency or rise-time of the e.j.p., or in the amplitude-frequency distribution of s.j.ps, suggest that it cannot be attributed to changes in the membrane properties of the muscle cells. It seems likely, therefore, that the reduction of e.j.p. amplitude by dopamine reflects a reduction in transmitter release.

The amount of transmitter released during stimulation of the entire neural input to a tissue could be reduced by a generalized change in axonal membrane potential (Castillo & Katz, 1954) or by failure of axonal impulse conduction (Krnjević & Miledi, 1958) as well as by a specific effect on the release process. However no evidence for involvement of the first two mechanisms was obtained in this study. At the somatic neuromuscular junction, alteration of axonal membrane potential produces reciprocal changes in e.p.p. amplitude and m.e.p.p. frequency (Castillo & Katz, 1954; Liley, 1956; Hubbard & Willis, 1962; 1968). At the autonomic neuromuscular junction, the smallest s.j.ps cannot be detected above the baseline noise (Burnstock & Holman, 1962a), but it would be expected that any appreciable alteration in discharge frequency would be discernible. As the rate of occurrence of s.j.ps was not altered in the presence of dopamine, a change in axonal membrane potential seems unlikely. In many smooth muscle tissues, the e.j.p. recorded in any one muscle cell consists of components due to transmitter release from a number of axons (see Burnstock & Bell, 1974). An estimate of the number of axons (or groups of axons) responsible for the e.j.p. can be obtained by counting the number of stepwise reductions in e.j.p. amplitude as the stimulus voltage is reduced (Bell, 1969; Hirst, 1977). In a tissue such as the vas deferens, where the density of innervation is very high, small numbers of axonal contributions could probably be lost without producing a clear change in the e.j.p. However it seems likely that interference with axonal impulse conduction sufficient to reduce considerably e.j.p. amplitude would also be reflected in a decreased number of functional axon groups. This was not the case. Thus the most probable mechanism of action of dopamine in depressing transmission is one directly on the release process. In this context, Dun & Nishi (1974) found that dopamine reduces transmitter release from preganglionic terminals by a reduction of quantal content.

During a repetitive train of stimuli, the initial depression of e.j.p. amplitude was not maintained, due to an increased amount of facilitation in the presence of dopamine. This had the result that, at 1 Hz stimulation frequency, the amplitude of the fully-facilitated e.j.p. was little different from that seen under control conditions despite the profound reduction in amplitude of the first e.j.p. of the series. At a number of adrenergic neuromuscular junctions facilitation is minimal at stimulus frequencies of about 0.15 Hz and increases progressively as the frequency is increased (Burnstock *et al.*, 1964; Bell, 1969; Bennett, 1973; Hirst, 1977). Thus the extent to which dopamine depresses transmission would be expected to be inversely related to stimulus frequency. This was illustrated by the fact that at 0.4 Hz, when facilitation is less pronounced than at 1.0 Hz, the depression of e.j.p. amplitude by dopamine during repetitive stimulation was correspondingly more maintained. Such a relationship explains the inverse frequency-dependence for depression by α-adrenoceptor agonists of both transmitter overflow and mechanical responses to nerve stimulation which has been seen over the range 0.1 to 10 Hz in a variety of preparations (Hughes, 1972; Armstrong & Boura, 1973; Vizi, Somogyi, Hodházy & Knoll, 1973; Starke, Endo & Taube, 1975).

The amplitude of the e.j.p. evoked by a single impulse was not reduced by the concentration of phentolamine used in the present experiments, despite abundant evidence that the excitatory innervation of the guinea-pig vas deferens is adrenergic (see, for in-

stance, Burnstock & Holman, 1962b; Swedin, 1971; Burnstock & Bell, 1974). This is in agreement with several reports in the literature which have previously indicated that phentolamine also has little depressant effect on neurogenic contractions of this tissue (Swedin, 1971; Ambache & Zar, 1971; see also Westfall, 1977). On the other hand, the depressant effect of dopamine on amplitude of a single e.j.p. was completely antagonized by phentolamine, supporting the view that this effect was mediated through activation of presynaptic α-adrenoceptors.

During repetitive stimulation phentolamine as well as dopamine increased the amount of facilitation which occurred. Increased transmitter overflow in the presence of α-adrenoceptor antagonists is well documented for a variety of adrenergically innervated tissues (Brown & Gillespie, 1957; Langer, 1970; Farnebo & Hamberger, 1971 and others) and recently several α-adrenoceptor antagonists have been shown to prolong the facilitation process in the mouse vas deferens (Bennett & Middleton, 1975). Thus in terms of their enhancement of transmitter release during repetitive stimulation both dopamine and phentolamine appear to exert similar effects. Furthermore, when dopamine was applied in the presence of phentolamine these effects were seen to be additive rather than antagonistic, suggesting that it was independent of any action of the drugs on α-adrenoceptors. It is of interest that Hurst, Marshall & Nasmyth (1979) have recently described an enhancing effect of dopamine on transmitter overflow in mouse vas deferens which also appears to be independent of α-adrenoceptors.

Proposals have been made that the presynaptic α-adrenoceptor has a physiological role in feedback modulation of adrenergic transmission (see Langer, 1974; Starke, 1977; Westfall, 1977 for reviews). The experimental basis of these proposals is the enhancement of transmitter release in the presence of α-adrenoceptor antagonists. In view of the evidence presented in this paper that such an effect might occur by an action of α-adrenoceptor antagonists at a site other than an α-adrenoceptor, it is desirable to obtain further data on the specificity of action of these compounds.

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