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# The pharmacological nature of the response of the reserpinized guinea-pig vas deferens to postganglionic nerve stimulation

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1. Intracellular recordings from single smooth muscle cells of the guinea-pig vas deferens have been made after depletion of neuronal stores of noradrenaline by chronic reserpine treatment.

2. Junctional responses to postganglionic hypogastric nerve stimulation were depressed, but large excitatory junction potentials (EJPs) and action potentials could still be evoked by stimulation at 1–2 c/s.

3. These junctional responses were not reduced by the presence of hyoscine ($5 \times 10^{-7}$ g/ml.), but were greatly depressed by the presence of bretylium ($2 \times 10^{-6}$ g/ml.).

4. Even in the presence of bretylium, junctional responses leading to action potentials could still be evoked by using high voltages of stimulation.

5. It is concluded that when neuronal noradrenaline stores have been depleted by reserpine some noradrenaline is still available for release by nerve stimulation.

6. It is further concluded that if the non-adrenergic component of the response of the guinea-pig vas deferens to nerve stimulation is cholinergic, this component is resistant to blockade by hyoscine.

Both pharmacological and chemical evidence suggests that the major component of the motor innervation of the guinea-pig vas deferens is adrenergic (Huković, 1961 ; Burnstock & Holman, 1961 ; Sjöstrand, 1965). However, the tissue contains both choline acetyltransferase (Ohlin & Stromblad, 1963) and acetylcholinesterase, which is localized in nerve bundles (Jacobowitz & Koelle, 1965 ; Bell & McLean, 1967), and responses to postganglionic nerve stimulation are potentiated by anticholinesterase treatment (Birmingham, 1966 ; Bell, 1967). In contrast, atropine and hyoscine produce little or no reduction of responses to stimulation (Ohlin & Stromblad, 1963 ; Della Bella, Benelli & Gandini, 1964).

The potentiating effect of anticholinesterase agents has been explained in two ways: by the possibility that endogenous acetylcholine is involved in the physiological release of noradrenaline from adrenergic nerves (Burn & Rand, 1959 ; Burn

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& Weetman, 1963; Burn, 1966), and by the possibility that the excitatory nerve supply contains both adrenergic and cholinergic nerve fibres (Birmingham, 1966; Bell, 1967). In view of the lack of effect of atropine and hyoscine on the response of the vas deferens to nerve stimulation, acceptance of the second proposal relies on the assumption that any cholinergic system present is resistant to blockade by these agents.

In order to investigate this possibility, the effect of hyoscine on transmission to the guinea-pig vas deferens has been investigated following depletion of the tissue noradrenaline with reserpine (Sjöstrand, 1962; Burnstock & Holman, 1962), as under these conditions any cholinergic component of the response to nerve stimulation would be expected to be greater than normal.

## Methods

Isolated hypogastric nerve—vasa deferentia preparations from adult male guinea-pigs were mounted in modified Krebs solution (Huković, 1961), at 36°–37° C, and prepared for intracellular micro-electrode recording from the cells of the longitudinal muscle layer and for postganglionic nerve stimulation as described previously (Bell, 1967). Drugs used were bretylium tosylate (Darenthin, Wellcome); hyoscine hydrobromide (BDH); and reserpine (Serpasil, Ciba). Reserpine was administered in doses of 5 mg/kg intraperitoneally, 48 and 24 hr before the experiment. The other drugs were applied to the isolated tissue via the flow of bathing solution.

## Results

As described by previous workers (Burnstock & Holman, 1962), the excitatory junction potentials (EJPs) recorded from the vas deferens in response to hypogastric stimulation after reserpine treatment were small in amplitude and showed a slow rate of facilitation in comparison to EJPs recorded from normal vasa deferentia. A typical response to a train of stimulating pulses at 1 c/s is illustrated in Fig. 1a. Reserpine-induced depression of tranmission was further evidenced by the fact that

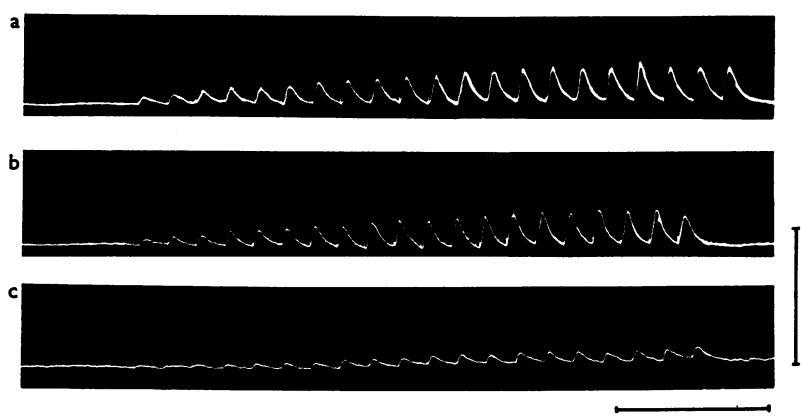


FIG. 1. Excitatory junction potentials recorded from the reserpinized guinea-pig vas deferens in response to postganglionic stimulation of the hypogastric nerve at 1 c/s and 59 V. a, Control and b, neighbouring cell 30 min after application of hyoscine ($5 \times 10^{-7}$ g/ml.); c, another neighbouring cell 30 min after subsequent application of bretylium ($2 \times 10^{-6}$ g/ml.). Calibrations: 5 sec and 25 mV.

the magnitude of the stimulation parameters necessary to produce muscle cell depolarization sufficient to initiate action potentials was considerably higher than in normal vasa deferentia. At a stimulation frequency of 1 c/s, the minimum stimulation voltage necessary for the initiation of action potentials was generally 10–20 fold greater than normal (Fig. 2a, Table 1: compare Fig. 1 of Bell, 1967).

Application of hyoscine ($5 \times 10^{-7}$ g/ml.), even after contact with the tissue for 60 min, had no effect on either the pattern and amplitude of EJPs evoked by stimulation at a particular voltage (Fig. 1b) or the magnitude of the stimulation parameters necessary for initiation of action potentials (Fig. 2b; Table 1). In contrast bretylium ($2 \times 10^{-6}$ g/ml.) caused a reduction in the amplitude of EJPs (Fig. 1c), and a large increase in magnitude of the stimulation parameters necessary for firing action potentials. Thus with a constant frequency of stimulation, the voltage needed to cause firing was raised (Table 1), while with a constant voltage, the stimulation frequency necessary to cause firing rose (Fig. 2c). However, the blocking effect of bretylium, either before or after hyoscine, was never total, and EJPs and action potentials could still be elicited in all cells impaled following

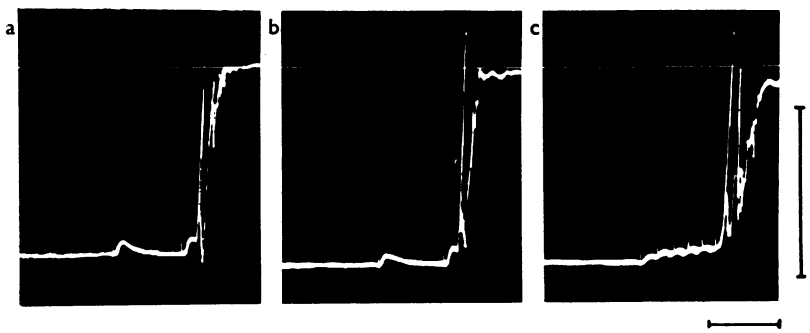


FIG. 2. Initiation of action potentials in the reserpinized guinea-pig vas deferens by post-ganglionic stimulation at constant voltage (60 V). a, Control conditions; b, 40 min after application of hyoscine ($5 \times 10^{-7}$ g/ml.); c, 30 min after subsequent application of bretylium ($2 \times 10^{-6}$ g/ml.).

TABLE 1. *Reserpine pretreated guinea-pig vas deferens: effects of hyoscine* ($5 \times 10^{-7}$ *g/ml.) and bretylium* ($2 \times 10^{-6}$ *g/ml.) on the voltage necessary to initiate action potentials (APs) during trains of post-ganglionic hypogastric stimulation*

| Experiment | Treatment | No. cells | Stim. freq. (c/s) | Minimum stim. voltage for initiation of APs |
|---|---|---|---|---|
| 1 | Control | 16 | 1 | 91–92 |
| | Hyoscine | 18 | 1 | 91–93 |
| | Bretylium | 5 | 1 | 148–149 |
| 2 | Control | 10 | 1 | 60–62 |
| | Hyoscine | 9 | 1 | 61–61·5 |
| | Bretylium | 12 | 1 | 121–124 |
| 3 | Control | 6 | 2 | 42–43 |
| | Hyoscine | 10 | 2 | 42–42·5 |
| | Bretylium | 5 | 2 | 81–88 |
| 4 | Control | 10 | 2 | 60–61 |
| | Bretylium | 12 | 2 | 120–123 |
| | Hyoscine | 16 | 2 | 123–123·5 |

The frequency of stimulation was kept constant in each experiment.

bretylium treatment, provided that either the frequency or voltage of stimulation was sufficiently high (Fig. 1c, 2c).

## Discussion

It is well known that chronic reserpine treatment causes depression of both mechanical and junctional responses of the vas deferens to hypogastric stimulation (Huković, 1961 ; Burnstock & Holman, 1962 ; Bhargava, Kar & Parmar, 1965). This depression is associated with pronounced depletion of tissue stores of noradrenaline (Sjöstrand, 1962). Thus it would be expected that, if acetylcholine were concerned with transmission, following reserpine treatment the major portion of any residual response to stimulation would be attributable to acetylcholine.

The present results showed that, following reserpine, the junctional responses obtained were greatly reduced by bretylium and not affected by hyoscine. The effect of bretylium indicates that after reserpinization there still exist stores of noradrenaline which can be released by the nerve impulse. Similar conclusions have been drawn from results obtained on other tissues using radiotracer and histochemical techniques (Fischer & Kopin, 1964 ; Fuxe & Sedvall, 1964). However, bretylium was never effective in completely abolishing responses to stimulation, suggesting the participation in transmission of a transmitter other than noradrenaline ; and by the pharmacological and histochemical evidence this substance would be expected to be acetylcholine. If this is accepted, then the lack of effect of hyoscine on the junctional responses both before and after bretylium must be assumed to indicate that the nervously released acetylcholine is resistant to antagonism by hyoscine. A similar situation appears to exist with regard to the cholinergic motor innervation of the bladder of numerous species (Dale & Gaddum, 1930 ; Ambache, 1955 ; Ursillo, 1961 ; Burnstock & Campbell, 1963 ; Huković, Rand & Vanov, 1965 ; Chesher & Thorpe, 1965). Thus the results of this investigation suggest that the lack of effect of agents blocking muscarinic receptors on the motor response of the guinea-pig vas deferens cannot be used as evidence against the presence of functional cholinergic nerves.

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## REFERENCES

AMBACHE, N. (1955). The use and limitations of atropine for pharmacological studies on autonomic receptors. *Pharmac. Rev.*, **7**, 467–494.

BELL, C. (1967). An electrophysiological study of the effects of atropine and physostigmine on transmission to the guinea-pig vas deferens. *J. Physiol., Lond.*, **189**, 31–42.

BELL, C. & MCLEAN, J. R. (1967). Localization of norepinephrine and acetylcholinesterase in separate neurons supplying the guinea-pig vas deferens. *J. Pharmac. exp. Ther.*, **157**, 69–73.

BHARGAVA, K. P., KAR, K. & PARMAR, S. S. (1965). Independent cholinergic and adrenergic mechanisms in the guinea-pig isolated vas deferens preparation. *Br. J. Pharmac. Chemother.*, **24**, 641–650.

BIRMINGHAM, A. T. (1966). The potentiation by anticholinesterase drugs of the responses of the guinea-pig isolated vas deferens to alternate preganglionic and postganglionic stimulation. *Br. J. Pharmac. Chemother.*, **27**, 145–157.

BURN, J. H. (1966). Section V. Adrenergic transmission. Introductory remarks. *Pharmac. Rev.*, **18**, 459–470.

BURN, J. H. & RAND, M. J. (1959). Sympathetic postganglionic mechanism. *Nature, Lond.*, **184**, 163–165.

BURN, J. H. & WEETMAN, D. F. (1963). The effect of eserine on the response of the vas deferens to hypogastric nerve stimulation. *Br. J. Pharmac. Chemother.*, **20**, 74–82.

BURNSTOCK, G. & CAMPBELL, G. (1963). Comparative physiology of the vertebrate autonomic nervous system II. Innervation of the urinary bladder of the ringtail possum (*Pseudocheirus peregrinus*). *J. exp. Biol.*, **40**, 421–436.

BURNSTOCK, G. & HOLMAN, M. E. (1961). The transmission of excitation from autonomic nerve to smooth muscle. *J. Physiol., Lond.*, **155**, 115–133.

BURNSTOCK, G. & HOLMAN, M. E. (1962). Effect of denervation and of reserpine treatment on transmission at sympathetic nerve endings. *J. Physiol., Lond.*, **160**, 461–469.

CHESHER, G. B. & THORP, R. H. (1965). Atropine resistance to intrinsic nerve stimulation of the guinea-pig bladder. *Br. J. Pharmac. Chemother.*, **25**, 288–294.

DALE, H. H. & GADDUM, J. H. (1930). Reactions of denervated voluntary muscle, and their bearing on the mode of action of parasympathetic and related nerves. *J. Physiol., Lond.*, **70**, 109–143.

DELLA BELLA, D., BENELLI, G., & GANDINI, A. (1964). Eserine and autonomic nervous control of guinea-pig vas deferens. *J. Pharm. Pharmac.*, **16**, 779–787.

FISCHER, J. E. & KOPIN, I. J. (1964). Evidence for intra- and extraneuronal reserpine resistant norepinephrine pools. *Fedn Proc.*, **23**, 349.

FUXE, K. & SEDVALL, G. (1964). Histochemical and biochemical observations on the effect of reserpine on noradrenaline storage in vasoconstrictor nerves. *Acta physiol. scand.*, **61**, 121–129.

HUKOVIĆ, S. (1961). Responses of the isolated sympathetic nerve—ductus deferens preparation of the guinea-pig. *Br. J. Pharmac. Chemother.*, **16**, 188–194.

HUKOVIĆ, S., RAND, M. J. & VANOV, S. (1965). Observations on an isolated innervated preparation of rat urinary bladder. *Br. J. Pharmac. Chemother.*, **24**, 178–188.

JACOBOWITZ, D. & KOELLE, G. B. (1965). Histochemical correlations of acetylcholinesterase and catecholamines in postganglionic autonomic nerves of the cat, rabbit and guinea-pig. *J. Pharmac. exp. Ther.*, **148**, 225–237.

OHLIN, P. & STRÖMBLAD, B. C. R. (1963). Observations on the isolated vas deferens. *Br. J. Pharmac. Chemother.*, **20**, 299–306.

SJÖSTRAND, N. O. (1962). Effect of reserpine and hypogastric denervation on the noradrenaline content of the vas deferens and the seminal vesicle of the guinea-pig. *Acta physiol. scand.*, **56**, 376–380.

SJÖSTRAND, N. O. (1965). The adrenergic innervation of the vas deferens and the accessory male genital glands. *Acta physiol. scand.*, **65**, supple. 257.

URSILLO, R. C. (1961). Investigation of certain aspects of atropine resistant nerve effects. *J. Pharmac. exp. Ther.*, **131**, 231–236.

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