J. Physiol. (1973), **231**, *pp.* 251–270
With 7 text-figures
Printed in Great Britain

AN INHIBITION OF POST-GANGLIONIC MOTOR TRANSMISSION IN THE MAMMALIAN VAS DEFERENS BY D-LYSERGIC ACID DIETHYLAMIDE

BY N. AMBACHE, LINDA P. DUNK, J. VERNEY AND M. ABOO ZAR

From the Medical Research Council Department of Physiology, Royal College of Surgeons of England, Lincoln's Inn Fields, London WC2A 3PN

(*Received 7 September 1972*)

SUMMARY

1. Under certain conditions D-lysergic acid diethylamide (LSD), 10^{-9}–10^{-6} g/ml., exerted an immediate, prolonged and slowly reversible inhibitory effect upon the post-ganglionic motor transmission in desheathed guinea-pig vas deferens preparations.

2. The most critical factor influencing this action of LSD appeared to be the train length. With short trains of less than 4 or 5 pulses the twitch inhibition produced by LSD was often total. With longer trains (5–20 pulses), the degree of inhibition declined with increase in train length. These results suggest the existence of two components in the motor response to post-ganglionic stimulation, distinguished by their susceptibility to LSD.

3. The inhibition of the LSD-susceptible component was related to the dose of LSD in the range 10^{-9}–10^{-6} g/ml., reaching a maximum at 0·5–1×10^{-6} g/ml. The response remnants elicited by trains of more than 5 pulses under these conditions could not be reduced further by a ten- to twenty-fold increase in LSD concentration to 10^{-5} g/ml. and were in fact slightly potentiated.

4. The inhibition of post-ganglionic motor transmission by LSD was not explicable on the basis of an α-adrenoceptor blockade because it was not associated with any reduction in motor responses to noradrenaline.

5. The use of propranolol excluded mediation of the LSD-inhibition by β-adrenoceptors.

6. The LSD effect was not due to a non-specific smooth muscle depression because it was not associated with any reduction in motor responses to acetylcholine, ATP or bradykinin.

7. The inhibitory effect of LSD on post-ganglionic transmission resembled that of noradrenaline in that it was antagonized by phentolamine;

another α-adrenoceptor blocking agent, phenoxybenzamine, was less effective than phentolamine in this respect.

8. The LSD-inhibition was obtained in preparations taken from reserpinized guinea-pigs.

9. The inhibition of motor transmission in the vas deferens by LSD was confirmed in rats, *Meriones shawii* and rabbits.

10. The inhibition of post-ganglionic transmission by LSD was unrelated to its ability to antagonize 5-hydroxytryptamine (5-HT), to which the longitudinal muscle of the guinea-pig vas deferens is insensitive. The more potent 5-HT antagonists, methysergide and BOL 148 were either virtually inactive or considerably weaker than LSD.

INTRODUCTION

Reasons have been given in our earlier publications (Ambache & Zar, 1971; Ambache, Dunk, Verney & Zar, 1972*a*, *b*) for believing that the adrenergic fibres in the vas deferens subserve not a motor but an inhibitory role and that post-ganglionic motor transmission in this organ is mediated by an unknown neurotransmitter.

During these and subsequent investigations we have been puzzled by a discrepancy in the effect upon this transmission of some drugs commonly used as 5-HT antagonists. Tryptaminergic motor transmission could be excluded because the longitudinal muscle of the guinea-pig vas deferens was insensitive to 5-HT and because the motor transmission was not antagonized by two of the most powerful 5-HT blocking agents available, methysergide and 2-bromolysergic acid diethylamide (BOL 148). On the other hand, we have now made the unexpected observation that, unlike methysergide and BOL 148, lysergic acid diethylamide (LSD) in low concentrations exerts a profound inhibitory effect upon post-ganglionic motor transmission in the vas deferens of several species and reveals the existence of two components in the motor transmission, distinguishable by their susceptibility to LSD.

A pharmacological analysis of the effects of LSD on motor transmission in the vas deferens has been carried out and is now described. A preliminary account of some of the results has appeared elsewhere (Ambache, Dunk, Miall & Zar, 1971).

METHODS

Most of the experiments were performed on guinea-pig vas deferens preparations; the animals, chiefly albinos weighing 0·3–1 kg, were killed by concussion. A few experiments were also conducted on vasa from albino Wistar rats, *Meriones shawii* and rabbits. The method for desheathing and setting up the isolated vas deferens preparations *in vitro* in a 2 ml. organ-bath at 35° C and a full description of essential

experimental details, including the composition of the Krebs–Henseleit solution, have been given previously (Ambache & Zar, 1971). In particular, the reader is referred to the earlier paper for procedural details of the intermittent field stimulation with a low output impedance stimulator, now modified to deliver up to 40 V at 800 mA, and for the evidence, given in Fig. 2 of that paper and based upon the use of a ganglion blocker, that the twitch responses were due entirely to excitation of post-ganglionic fibres. In the present experiments stimulation, pulse width, intermittence and voltage were kept constant throughout each experiment: the pulse width, at 0·1, 1 or 2 msec; the intermittence, at 33 or 60 sec intervals; and the voltage, at a value slightly above that required to produce maximal contractions due entirely to nerve stimulation without direct muscle excitation, as shown by tetrodotoxin (see Ambache & Zar, 1971, Figs. 1 and 2). The contractions of the longitudinal muscle were recorded isometrically by means of transducers (limit of sensitivity, 10 or 25 g) at a resting tension of *ca.* 0·3 g, achieved by setting up the preparation at an initial tension of 0·5 g.

To avoid undesirable effects attributable to fatigue or depletion after long trains of pulses, in some experiments, e.g. Fig. 3, one vas was reserved for stimulation with 1–5 pulses, and the contralateral vas was used for longer trains.

Reserpinization. The animals received two injections of reserpine phosphate solution (5 mg/ml. in distilled water) in a daily dose of 5 mg/kg, s.c. on the first day and I.P. on the second, and were killed on the third day. This dosage of reserpine is 5 times greater than that recommended by Sjöstrand (1962) to achieve a 'total' depletion of noradrenaline in the guinea-pig vas deferens. More recently, Wakade & Krusz (1972) have shown that a single 5 mg/kg dose of reserpine I.P. produced a 98·8% depletion of noradrenaline in this tissue; a second dose of 3 mg/kg next day increased the depletion to 99·7%.

Drugs. With the exception of bradykinin and ergocristine bases, all dosages refer to the respective salts, namely: acetylcholine chloride, adenosine-5′-triphosphate disodium salt (ATP), D-2-bromolysergic acid diethylamide hydrogen tartrate (BOL 148), dihydroergotamine methane sulphonate, ergometrine hydrogen maleate ('ergonovine maleate', Sigma Chemical Co.), ergotamine tartrate, 5-hydroxytryptamine creatinine sulphate, isoprenaline hydrochloride, methysergide hydrogen maleate, L-noradrenaline bitartrate, phenoxybenzamine hydrochloride, phentolamine methane sulphonate, propranolol hydrochloride, reserpine phosphate (kindly supplied by Dr A. J. Plummer, Ciba, Summit, N.J., U.S.A.) and tyramine hydrochloride.

We are indebted to Dr E. R. Evans, Sandoz Ltd, London, for samples of methylergometrine (D-lysergic acid-hydroxybutylamide-2) hydrogen maleate and of crystalline D-lysergic acid diethylamide tartrate (LSD-25) of empirical formula

$$[C_{15}H_{15}N_2CON(C_2H_5)_2]_2.C_4H_6O_6.$$

The comparison of the dibasic LSD-25 with the other lysergamides, most of which were available as monobasic hydrogen maleate or hydrogen tartrate salts, is rendered easier if the 'effective molecular weight' of LSD-25 is taken as consisting of 1 mole of lysergic diethylamide + ½ mole of tartaric acid.

RESULTS

Effect of LSD on guinea-pig vas deferens

In the absence of electrical stimulation LSD, in doses ranging from 10^{-9} to 10^{-5} g/ml., did not produce any visible alteration in the resting tension of the preparations.

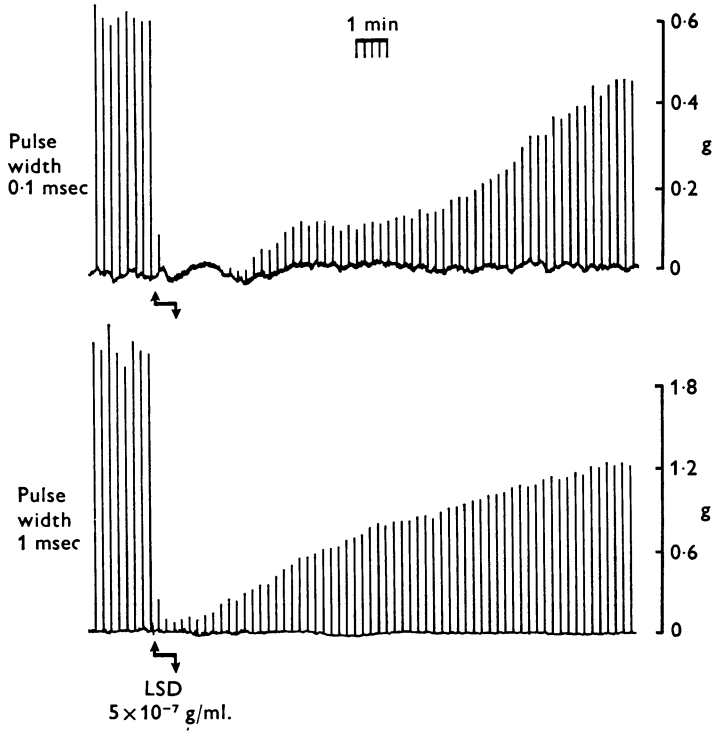

Fig. 1. Prolonged inhibition of post-ganglionic motor transmission by LSD in guinea-pig vas deferens. Parallel preparations from the same animal, both stimulated at 1 min intervals with trains of 4 pulses delivered at 10 Hz; pulse width, 0·1 msec in the upper and 1 msec in the lower preparation; voltage kept constant for each preparation. Between the arrows, LSD, 5×10^{-7} g/ml., was administered for 3 min. Note the pronounced inhibition and slow recovery in both experiments. Isometric tension calibrations in g; the zero level represents a basal resting tension of *ca.* 0·3 g (see Methods). Further explanation in text.

Inhibition of electrically evoked twitches by LSD

Desheathing of the guinea-pig vas deferens is known to render it ganglion-free; the effect of field stimulation with pulses of 0·1–2 msec is then confined to excitation of post-ganglionic nerve fibres, resulting in

twitches initiated by the release of an unknown motor transmitter (Ambache & Zar, 1971). The excitability curves illustrated in Fig. 1 of Ambache & Zar's (1971) paper suggest the existence of two sets of post-ganglionic fibres: one set selectively excitable at pulse widths of 0·1–0·4 msec, the other set requiring pulse widths of 0·5–2 msec.

In most of the following experiments the effect of LSD was studied at pulse widths of 1 or 2 msec, but many of the results were confirmed in additional, duplicate experiments in which a pulse width of 0·1 or 0·2 msec

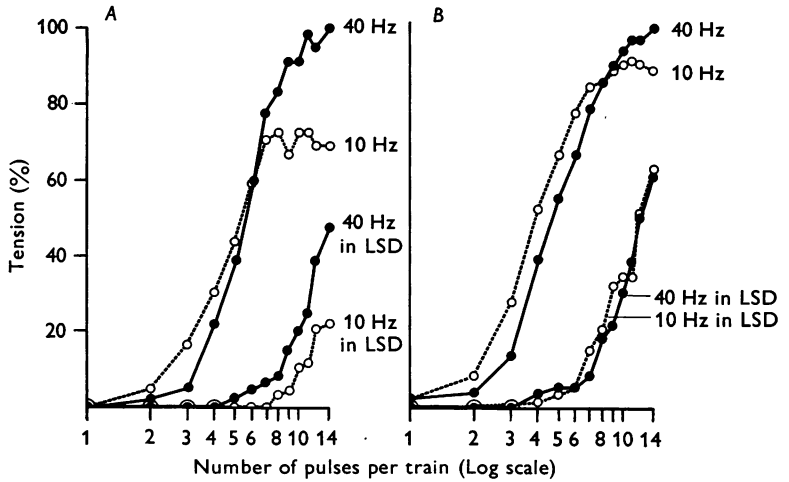

Fig. 2. Influence of train length and frequency on inhibition of post-ganglionic motor transmission by LSD in guinea-pig vas deferens. Parallel desheathed preparations from the same animal, stimulated at 1 min intervals with trains of 1–14 pulses of constant voltage, either at 10 Hz (○--○) or at 40 Hz (●--●). Pulse width: 0·1 msec for preparation *A* and 1 msec for *B*; in both cases all tensions have been expressed as the % of the maximum tension which was recorded with 14 pulses at 40 Hz. The preparations were exposed to LSD, 5×10^{-7} g/ml., for at least 7 min before the LSD curves were obtained. In both experiments and at both frequencies the % inhibition produced by LSD declined as the number of pulses per train was increased. See text.

was also used, in order to confine the response to the more excitable set of post-ganglionic motor fibres and to test, selectively, the susceptibility to LSD of the response component due to these fibres.

Fig. 1 shows the prolonged inhibitory effect of LSD, 5×10^{-7} g/ml., in an experiment conducted in duplicate on preparations from the same animal. The two preparations were stimulated intermittently by trains of 4 pulses delivered at 10 Hz, but the pulse width was fixed at 0·1 msec for one preparation, so as to recruit selectively the more excitable set of motor

nerve fibres, and at 1 msec for the other preparation, in order to bring into play both sets of post-ganglionic fibres. The responses elicited at both pulse widths were susceptible to inhibition by LSD.

The inhibitory action of LSD appeared within 0·5–1·5 min of administration, and was fully developed within 2–8 min, higher doses requiring shorter exposures. Recovery of the twitches from this inhibition was slow; even after exposures lasting only 2–3 min, recovery took nearly 1 hr. The effect could be repeated by re-administration of LSD. After longer exposures and higher doses of LSD ($> 10^{-7}$ g/ml.) recovery was even slower and was incomplete for several hours.

Factors influencing the degree of inhibition produced by LSD

Pulse width. As shown in Figs. 1 and 2, the intensity of inhibition produced by LSD was more pronounced with 0·1 msec than with 1 msec pulses.

Train length. Fig. 2 also illustrates the effect of varying the train length upon the inhibitory action of LSD, 5×10^{-7} g/ml. Irrespective of frequency and pulse width, the degree of inhibition was most marked with single pulses and short trains of 2–4 pulses, and declined progressively as the train length was increased. Thus, at train lengths of up to 4 pulses LSD brought about a total extinction of the twitches, but when trains of 5–14 pulses were used, the LSD inhibition of motor transmission was only partial and diminished gradually as the number of pulses was increased.

Frequency. When the inhibitory action of LSD was examined at 5–100 Hz an interesting influence of frequency was observed. To illustrate this, two frequencies sufficiently wide apart were selected, namely 10 and 40 Hz. It can be seen from Fig. 2 that, in the absence of LSD, the motor response to a fixed number of pulses was greater at 10 Hz than at 40 Hz if the train length was less than six 0·1 msec or eight 1 msec pulses; when the train length exceeded these limits, the reverse was true. This Figure also shows that the larger motor responses elicited by trains of less than 6 or 8 pulses at 10 Hz were inhibited by LSD to a greater extent than the smaller responses elicited by the same number of pulses at 40 Hz; a similar result can be seen in the middle panel of Fig. 7. The differential effect of LSD was more marked at a pulse width of 0·1 msec (Fig. 2).

Depending upon the train length and frequency, LSD affected the motor transmission in one of the following three ways: (1) drastic inhibition, with 5 pulses at 10 Hz; (2) only slight depression, with 20 pulses at 40 Hz; or (3) sometimes, a slight potentiation, with 20 pulses at 10 Hz.

Concentration of LSD. The relationship between the degree of inhibition and the concentration of LSD was examined, first at short train lengths and then with longer trains of pulses. Two vasa from the same animal were

suspended in separate organ-baths; both preparations were stimulated at 1 min intervals with a varying number of 1 msec pulses of constant voltage and frequency fixed at 10 Hz. The results illustrated in the left-hand panel of Fig. 3 show that in the preparation stimulated with 1–5 pulses an appreciable inhibition of post-ganglionic motor transmission was produced even by the lowest concentration of LSD, 10^{-9} g/ml. With each rise in LSD concentration there was an increase in the degree of inhibition and the response curve was progressively shifted towards the right until a maximum of inhibition was reached at 10^{-6} g/ml., beyond

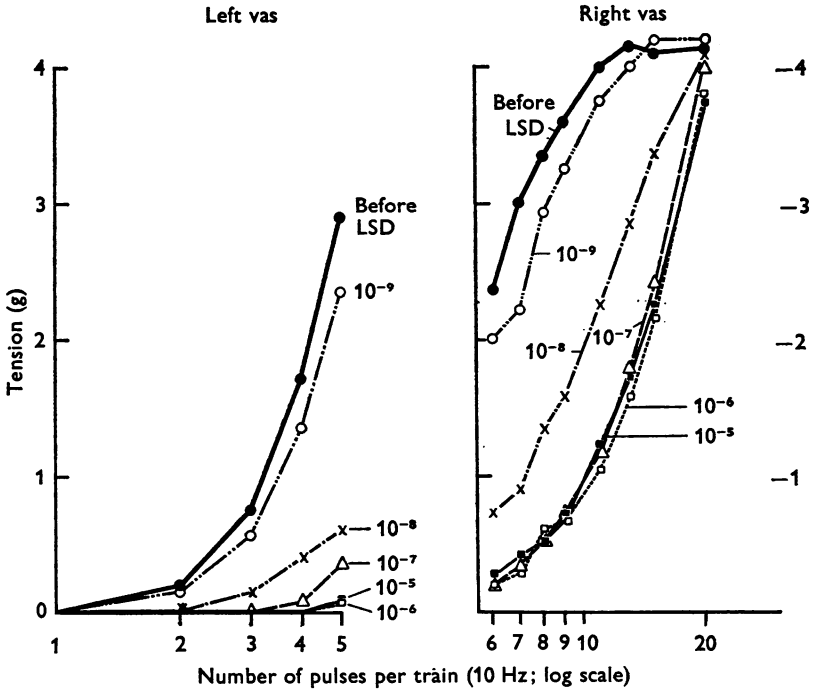

Fig. 3. Relationship between the dose of LSD and the degree of inhibition of the LSD-susceptible component in the motor response. Parallel vasa from the same guinea-pig, both excited at 1 min intervals with a varying number of 1 msec pulses of constant voltage, delivered at 10 Hz: the left vas was stimulated with 1–5 pulses and the right vas with longer trains of 6–20 pulses; before obtaining the response curve at each concentration of LSD a preliminary 8 min period of drug contact was allowed during which the preparations were stimulated with 2-pulse trains at 1 min intervals. Note increasing inhibition in the concentration range 10^{-9}–10^{-6} g/ml., with no further deepening of inhibition at 10^{-5} g/ml. The contribution of the LSD-resistant component to the motor response is insignificant with trains of < 5 pulses but grows spectacularly between 5 and 20 pulses.

Without LSD: ●—●; with LSD: ○—○ 10^{-9} g/ml., ×--× 10^{-8} g/ml., △--△ 10^{-7} g/ml., □--□ 10^{-6} g/ml., ■—■ 10^{-5} g/ml.

which there was no further shift of the curve to the right. The response to 2-pulse trains virtually disappeared at a concentration of 10^{-8} g/ml., and that to 3-pulse trains, at 10^{-7} g/ml. At a concentration of 10^{-6} g/ml. the response to 4-pulse trains had also virtually disappeared ($< 0{\cdot}6\,\%$), but there was still a $3{\cdot}5\,\%$ remnant of the response to 5-pulse trains. The next tenfold rise in the concentration of LSD, to 10^{-5} g/ml., failed to reduce this remnant any further. These results showed clearly that there was an LSD-susceptible process in the motor transmission of short trains, which was suppressed in proportion to the concentration of LSD; and another, LSD-resistant process, beginning to manifest itself at a train length of 5 pulses, which remained virtually unaffected on raising the concentration of LSD from 10^{-6} to 10^{-5} g/ml. It would appear that up to 4 pulses this LSD-resistant process normally contributed little or nothing to the motor responses; and with 5 pulses, only $3{\cdot}5\,\%$.

The right hand panel of Fig. 3 illustrates the results obtained with longer trains of 6–20 pulses on the contralateral vas. Again, LSD progressively shifted the response curve to the right, revealing the contribution of the LSD-resistant component to the total motor response. Some idea of the extent of this contribution can be gained from the response curve obtained after the elimination of the LSD-susceptible component at the maximally inhibiting concentration of LSD, 10^{-6} g/ml., beyond which there was no further shift of the response curve to the right. In Fig. 3 the contribution of this LSD-resistant but tetrodotoxin-susceptible component to the total motor response is seen to grow with train length.

The LSD-resistant component elicited by long trains of pulses was not due to noradrenaline release because it persisted in preparations pretreated for 10 min with phenoxybenzamine, 10^{-6} g/ml., and because it was obtained in vasa from reserpinized animals (see below). It was therefore quite distinct from an adrenergic, 'slow' component which has been described by Swedin (1971) in guinea-pig and rat vasa, using longer trains of 120–240 pulses. The mechanical responses in his experiments were biphasic, consisting of a fast twitch followed by a second, slow contraction; the latter was abolished by phentolamine and by pretreatment of the animals with reserpine. In the present experiments, records obtained at a high paper speed (240 mm/min) showed that even with 20 pulses the mechanical responses were always monophasic, and that phentolamine, 2×10^{-6} g/ml., did not reduce the height or duration of these responses; in fact there was some increase, both in height and in duration.

Pharmacological analysis of the inhibitory effect of LSD on post-ganglionic motor transmission

Exclusion of β-adrenoceptor mediation

In the guinea-pig vas deferens β-adrenoceptor stimulation with isoprenaline results in an inhibition of electrically induced twitches (Large, 1965). Ambache & Zar (1971) have found that this is associated with a depression of the response to smooth-muscle contracting agents and that both these effects of isoprenaline can be prevented by β-adrenoceptor blockade with propranolol, 10^{-6} g/ml. In the present experiments the inhibition of motor transmission by LSD remained unaffected after block of isoprenaline-induced inhibitions by propranolol, $1–2 \times 10^{-6}$ g/ml., as shown in Fig. 5. The results presented in the following section also exclude mediation of the LSD-inhibition by β-adrenoceptors.

The motor responses to noradrenaline and to other spasmogens remain unaffected during LSD-inhibition

The inhibition of motor transmission by LSD was not due to a direct depressant action on the smooth muscle fibres, because this inhibition was not associated with any reduction in the motor responses elicited by several directly acting muscle spasmogens; contractions elicited by acetylcholine, ATP, bradykinin or noradrenaline were either unchanged or augmented in the presence of LSD.

The interaction with noradrenaline can be seen in Fig. 4, which illustrates two experiments on different preparations of otherwise contrasting properties. There was, for example, a considerable difference in the sensitivity of the smooth muscle to the motor action of noradrenaline, injected during interruptions of stimulation: the noradrenaline sensitivity was high in the first experiment (2 μg/ml. in the top record) and low in the second (10–20 μg/ml. in the bottom record). The dose of LSD was the same in both experiments: 5×10^{-7} g/ml. In the first experiment a 2·5 min exposure to this dose of LSD produced a total extinction of the twitches, elicited at 33 sec intervals by trains of two 2-msec pulses at 10 Hz, without affecting the motor responses to noradrenaline, 2 μg/ml., administered during the last 30 sec of the exposure to LSD.

In the second preparation the twitches were elicited at 1 min intervals by trains of four 2-msec pulses at 10 Hz. Exposure to LSD, 5×10^{-7} g/ml., for 3 min produced an 80 % inhibition of the twitches but a potentiation, off scale, of the noradrenaline contractions elicited during the last 1 min of the exposure to LSD; this can be seen in Fig. 4, first with the 20 μg/ml. dose of noradrenaline and then again during the second exposure to LSD, with the 10 μg/ml. dose of noradrenaline.

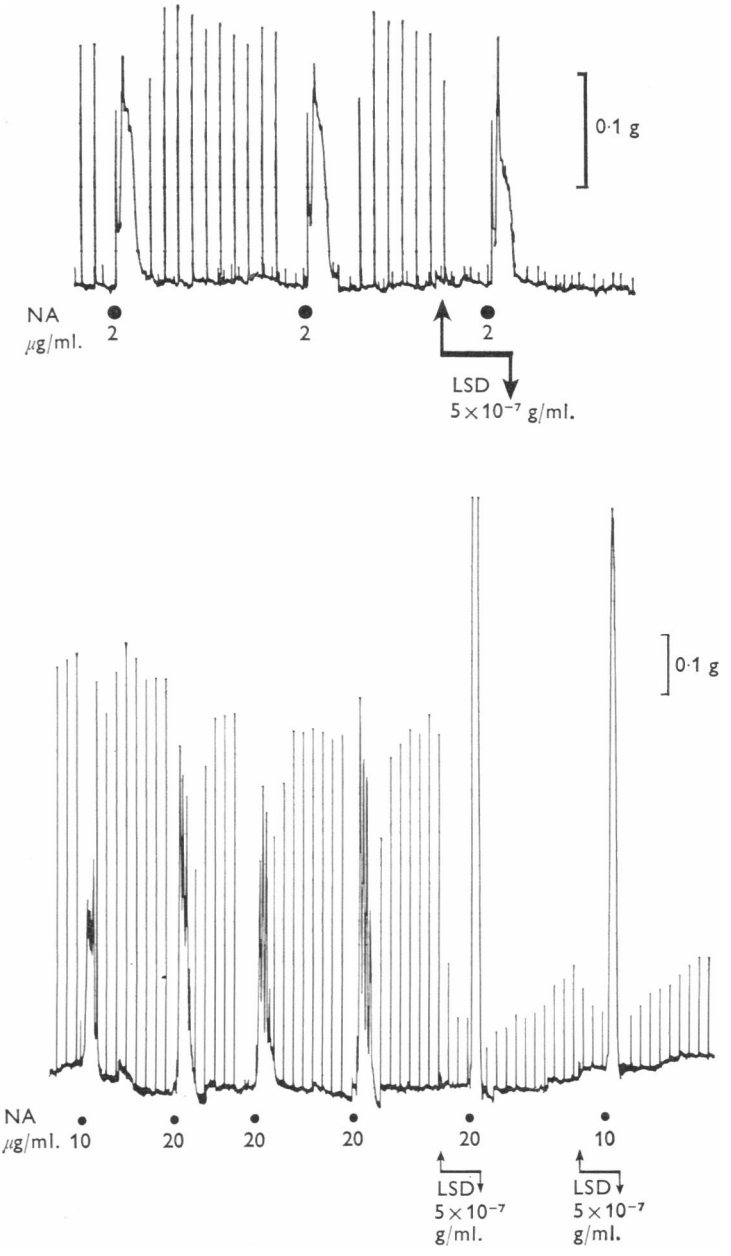

Fig. 4. For legend see facing page.

In further experiments the effect of LSD on the muscle response to other spasmogens was tested. In the experiment illustrated in Fig. 5 the inhibition of the twitches by LSD, 5×10^{-7} g/ml., was accompanied by a potentiation of the motor response to ATP, 5 μg/ml. Similar results were obtained when bradykinin was used, in preparations taken both from normal and from reserpinized guinea-pigs (Fig. 7). Contractions elicited by acetylcholine were also potentiated by LSD.

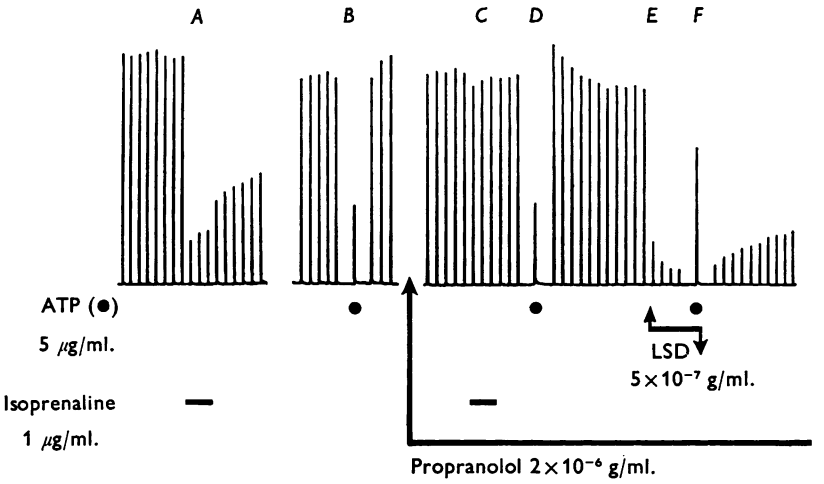

Fig. 5. The inhibitory effect of LSD is obtained after β-adrenoceptor blockade with propranolol; ATP contraction is potentiated during LSD inhibition. Guinea-pig vas deferens stimulated at 1 min intervals by trains of four 1 msec pulses (10 Hz) at constant voltage. Isoprenaline, 1 μg/ml., administered for 3 min at *A* before, and at *C* 32 min after, the introduction of propranolol, 2×10^{-6} g/ml., into the reservoir. At *E*, LSD, 5×10^{-7} g/ml., for 6 min. ATP was administered for 15 sec, during interruptions of electrical stimulation, at *B* and *D*, before LSD and at *F*, during the last 15 sec of the exposure to LSD.

Fig. 4. The LSD inhibition of post-ganglionic motor transmission is not associated with an α-blockade of noradrenaline (NA) contractions. Vasa from different guinea-pigs. Field stimulation at 10 Hz and 2 msec pulse width. Upper preparation: 2-pulse trains at 33 sec intervals. Lower preparation: 4-pulse trains at 1 min intervals. Voltage kept constant for each preparation.

At the dots, NA injected during interruptions of electrical stimulation: 2 μg/ml. for 30 sec in the upper, and 10 or 20 μg/ml. for 1 min in the lower record. The motor response to NA, administered again in the presence of LSD, 5×10^{-7} g/ml., when the twitch inhibition is fully developed, either remains unaltered (upper record) or undergoes considerable potentiation (lower record). Tension calibrations, 0·1 g.

Antagonism of the LSD inhibition by phentolamine

Phentolamine markedly reduced the degree of inhibition produced by LSD. The experiment illustrated in Fig. 6 shows the protective effect of phentolamine. In this experiment the two vasa from the same guinea-pig were suspended in separate organ-baths and were treated identically throughout, except that phentolamine was administered only to preparation *B*. In order to produce an inhibition comparable to that seen before

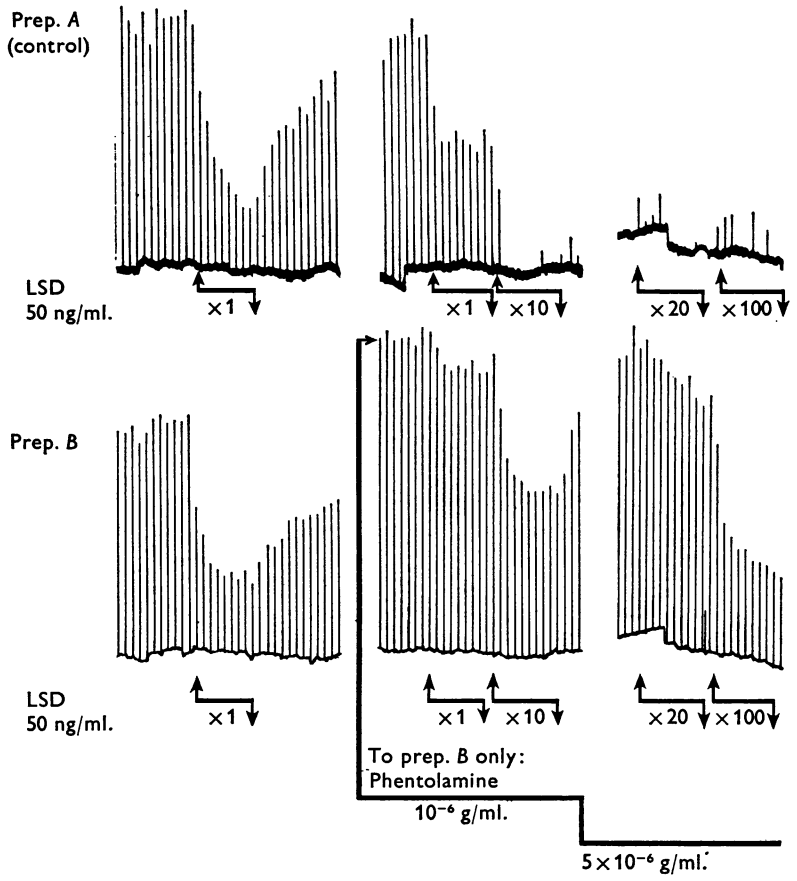

Fig. 6. Phentolamine antagonizes the inhibitory effect of LSD. Parallel vas deferens preparations from the same guinea-pig. Electrical stimulation at 1 min intervals by trains of two 1 msec pulses of constant voltage, delivered at 10 Hz. Between the arrows, LSD administered to both preparations in identical dosages, 50 ng/ml. or multiples thereof, as indicated. Preparation A: control, without phentolamine. Preparation B: phentolamine, 10^{-6} g/ml., administered 30 min before the middle panel and increased to 5×10^{-6} g/ml., 43 min before the last panel.

phentolamine, the dose of LSD had to be raised 10 times when the concentration of phentolamine was 10^{-6} g/ml., and 100 times when it was 5×10^{-6} g/ml.; this suggests the possibility of a competitive antagonism between phentolamine and LSD.

As reported earlier (Ambache & Zar, 1971) phentolamine, $1–2 \times 10^{-6}$ g/ml., often produced an augmentation of the twitch height, which can be seen in Figs. 6 and 7. The last panel of Fig. 6 shows that the fivefold increase in phentolamine concentration did not bring about any further augmentation of the twitches.

In other experiments, when phentolamine, 2×10^{-6} g/ml., was introduced into the organ-bath after the induction of inhibition by LSD, it partially reversed the inhibition; for instance, in one preparation the inhibition decreased from 89 to 61 % within 1 min.

Phenoxybenzamine, 10^{-6} g/ml., was considerably less effective than phentolamine in antagonizing the LSD inhibition.

Persistence of the inhibitory effect of LSD after reserpinization

In the guinea-pig vas deferens noradrenaline exerts an inhibitory effect upon post-ganglionic motor transmission; the noradrenaline inhibition can be prevented by phentolamine (Ambache & Zar, 1971). Similarly, tyramine and other indirectly sympathomimetic drugs which have the ability to release endogenous noradrenaline in the vas also inhibit motor transmission; this inhibitory effect of tyramine is abolished by phentolamine or by pre-treating the animals with reserpine (Ambache *et al.* 1972*b*). The possibility that LSD might act like tyramine was therefore examined; but it was found that the inhibitory action of LSD on motor transmission was not abolished by previous reserpinization of the guinea-pigs (Fig. 7). The effectiveness of reserpinization was indicated by the absence of twitch-inhibition when tyramine, 1–10 μg/ml., was administered (first panel). Nevertheless, motor transmission was profoundly inhibited by LSD, 5×10^{-8} g/ml., and, as found in normal vasa at certain train lengths, the degree of inhibition was greater at 10 Hz than at 40 Hz and there was no depression by LSD of contractions elicited by 1 min contacts with bradykinin, 0·25 μg/ml. (middle panel). The last panel of Fig. 7 shows that although the inhibitory effect of LSD was not abolished by reserpinization, it was still antagonized as usual by phentolamine.

The inhibitory activity of the LSD molecule is affected by 2-bromo and N-methyl substitution in the indole ring

Inhibition of post-ganglionic motor transmission could be obtained with alkaloid molecules in which the indole ring of the lysergic acid moiety is unsubstituted, e.g. with ergocristine, ergotamine or dihydroergotamine,

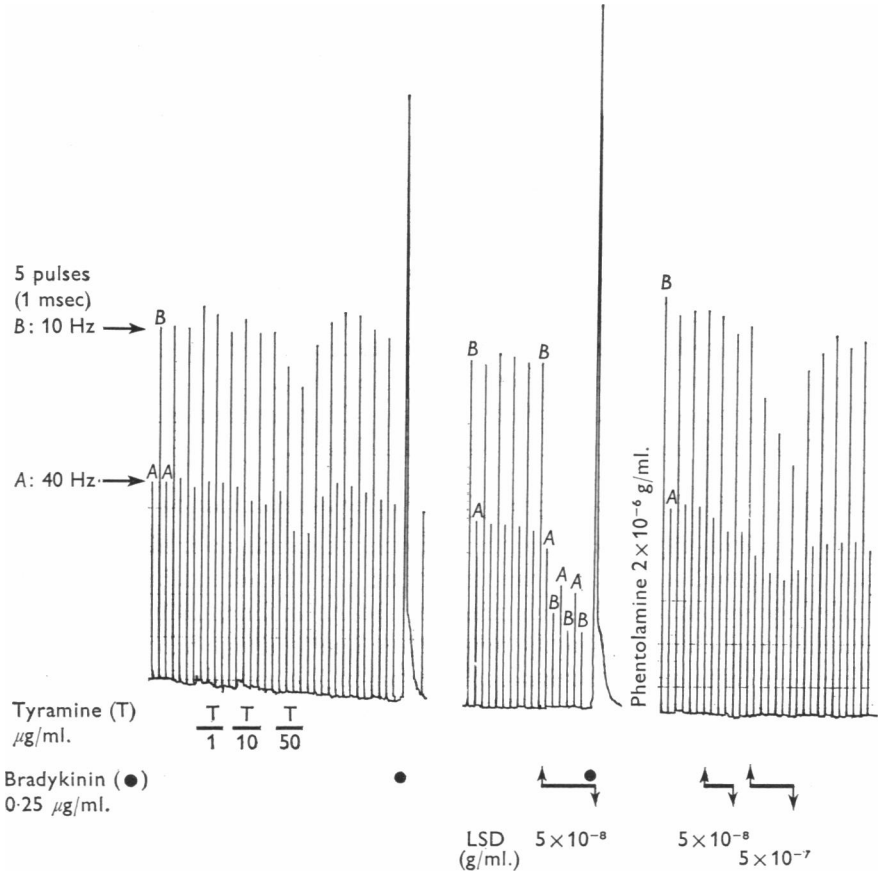

Fig. 7. Vas deferens preparation from a reserpinized guinea-pig; unlike the tyramine inhibition, the LSD inhibition is present as usual. The twitches were elicited by electrical stimulation at 1 min intervals with trains of five 1 msec pulses of constant voltage, delivered alternately at 40 Hz (*A*) or at 10 Hz (*B*); the responses were bigger at 10 Hz than at 40 Hz. At T, tyramine, 1, 10 or 50 μg/ml., for 4 min. The middle panel shows the pronounced twitch-inhibition produced by LSD, 5×10^{-8} g/ml.; note the much greater reduction of the larger responses elicited by 10 Hz than of the smaller responses elicited by 40 Hz. Phentolamine, 2×10^{-6} g/ml., introduced into the reservoir 26 min before the last panel, abolishes the inhibitory effect of LSD, 5×10^{-8} g/ml.; the phentolamine antagonism is partially overcome by a tenfold increase in the dose of LSD. At the dots, bradykinin contractions (1 min contacts) before and during exposure to LSD, showing the absence of smooth-muscle depression during the LSD inhibition. Explanation in text.

ergometrine and methylergometrine. On the other hand, it was found that small structural modifications to the LSD molecule, especially in the indole ring, greatly reduce or abolish its inhibitory activity. Thus, a considerable lowering of activity due to ring substitution was found with the 2-bromo derivative of LSD, BOL 148, which had negligible twitch-inhibiting activity in concentrations of 10^{-7}–10^{-5} g/ml. Likewise, *N*-methyl substitution in the indole ring, as in methysergide, greatly reduced or almost abolished the inhibitory activity; in concentrations of 10^{-7}–10^{-5} g/ml. this compound was found to be virtually inactive in four out of six experiments. The activity of methylergometrine, which has the same side chain as methysergide but no methyl substituent in the indole ring, was intermediate between that of LSD and of methysergide. These structure-activity relationships will be described more fully elsewhere.

Dihydroergotamine (DHE)

A complete extinction of vas deferens motor responses to transmural stimulation by this lysergic polypeptide was reported by Birmingham & Wilson (1963, Fig. 5), using very long trains of 375 pulses and concentrations of DHE (3×10^{-4} g/ml.) which apparently were non-specific because acetylcholine- and KCl-contractions were abolished as well. In the present experiments on four guinea-pig vasa, the inhibitory activity of DHE resembled that of LSD but, at short train lengths, was found to be about 10 times weaker on a molar basis. With 6 pulses, twitch height was halved as the DHE concentration was raised from 10^{-9} to 10^{-8} g/ml. and inhibitions of 78 and 89% were recorded at concentrations of 10^{-7} and $1{\cdot}75 \times 10^{-6}$ g/ml., respectively. The specificity of the effect of DHE was shown by the fact that bradykinin contractions were not depressed by these concentrations of DHE.

Other resemblances to the action of LSD, were: (1) the inhibitory effect of DHE was more marked at short train lengths, e.g. it was more than twice as great with 5–6 pulses as with 20 pulses; (2) it was obtained in the presence of propranolol, 2×10^{-6} g/ml., and was therefore not due to β-adrenoceptor stimulation; (3) phentolamine, 1–2×10^{-6} g/ml., prevented or reversed the inhibitory effect of DHE. The only difference from LSD was that DHE antagonized the contractile action of noradrenaline, as was to be expected from its well known α-adrenoceptor blocking property.

Other ergot alkaloids. Ergocristine, 5×10^{-7} g/ml., ergometrine, 10^{-7}–10^{-6} g/ml. and ergotamine, 10^{-6} g/ml., all produced strong inhibitions of twitches elicited by trains of 2–8 pulses at 10 Hz.

The effect of ergometrine and of ergotamine appeared to be maximal at 10^{-6} g/ml., because the twitch-remnants could not be reduced further even by a five- or tenfold increase in drug concentration.

LSD-inhibition in vasa from other species

LSD, 10^{-9}–10^{-5} g/ml., inhibited contractions of electrically stimulated vasa from the rat, rabbit and *Meriones shawii*. In rat vasa, which were investigated in greater detail, this inhibitory effect of LSD was similar to that observed in the guinea-pig in that it was most marked for single stimuli or for short trains of 2–5 pulses (10 Hz) and that it was antagonized by phentolamine, 10^{-6} g/ml; noradrenaline-induced contractions were slightly potentiated by LSD (5×10^{-7} g/ml.). The inhibition of the LSD-susceptible component reached a maximum at a concentration of LSD of 10^{-7} g/ml. In the rat vas, unlike the guinea-pig, the contraction elicited by single pulses or by short trains was never completely abolished by LSD. Similar results were obtained with DHE, except that noradrenaline contractions were abolished.

We have already considered (p. 263) whether LSD releases endogenous noradrenaline. In the rat vas, because the muscle fibres are more sensitive to the motor action of noradrenaline than they are in the guinea-pig, one of the effects of endogenous noradrenaline released by tyramine is to cause a reserpine-susceptible contraction (Ambache *et al.* 1972*b*). But LSD, unlike tyramine, never produced a contraction in normal rat vasa, in concentrations of 10^{-9}–10^{-5} g/ml.

Some differences between the rat and guinea-pig vas deferens were reported previously (Ambache *et al.* 1972*b*); several other differences were noted during the present investigation. The first was that, at supramaximal voltage, there was much less growth in the tension response of rat vasa as the pulse width was increased from 0·1 to 1 msec, suggesting that the motor nerve fibres are of more uniform excitability in the rat than in the guinea-pig vas deferens. The second was the much greater effectiveness in rat vasa of single pulses, which elicited 20–30 % of the maximum tension recorded with long trains at 10 Hz, as against 0–7 % in guinea-pig vasa. A third difference was the virtual insensitivity of rat vasa to ATP, 10–50 μg/ml.; this would seem to exclude ATP as a possible motor transmitter here.

DISCUSSION

The inhibitory action of LSD described in this paper is remarkable for its high potency and prolonged duration. This ability to inhibit a component in the post-ganglionic transmission to the vas deferens is shared to a lesser extent by other lysergic acid derivatives in which the indole ring is unsubstituted. Lysergic derivatives are endowed with multiple pharmacological properties, such as the ability to block α-adrenoceptors or to antagonize 5-HT. The present results indicate that neither of these two

properties is responsible for the inhibition of post-ganglionic motor transmission by LSD and that a new and hitherto unrecognized type of pharmacological activity has to be invoked to account for the suppression of the LSD-susceptible component in the post-ganglionic motor transmission. It is abundantly clear that this activity is quite unrelated to the 5-HT blocking property of LSD and its derivatives. As mentioned in the introduction, tryptaminergic motor transmission in the vas deferens was excluded in our previous paper (Ambache & Zar, 1971) on several counts, one of which was the observation, which we have confirmed again several times during this investigation (not reported in Results), that the longitudinal smooth muscle in this organ is insensitive to 5-HT. The present findings with methysergide and BOL 148 corroborate this conclusion; both these drugs are known to be 4–5 times more powerful than LSD as 5-HT antagonists in several smooth muscle preparations (Gyermek, 1961), yet they usually produce little or no inhibition of post-ganglionic motor transmission in the vas deferens.

The selection, for the present investigation, of the biethylamide derivative of lysergic acid, which is devoid of adrenoceptor-blocking properties, bypasses the pitfalls which are encountered if ergotamine or dihydroergotamine is used. The fact that these two drugs, though weaker than LSD, possess the ability to inhibit the motor transmission in the vas deferens (Boyd, Chang & Rand, 1960; Birmingham & Wilson, 1963) has been generally attributed to their well known α-adrenoceptor blocking property. This misconception has been removed in the present experiments by the use of LSD, which exerts an inhibitory action similar to that of ergotamine and dihydroergotamine on the motor transmission in the vas, but does so without producing any block of α-adrenoceptors in the muscle. The finding that the inhibitory effects of LSD and of dihydroergotamine are both antagonized by phentolamine emphasizes their similarity and suggests an identical site for this common action. The competitive nature of the antagonism between phentolamine and LSD is suggested by the observation that this antagonism was overcome on raising the dose of LSD (Fig. 6).

The use of LSD has revealed the existence of two distinct components in the monophasic motor response evoked by post-ganglionic stimulation with 1–20 pulses; only one of these components is suceptible to inhibition by LSD. When we have a better understanding of its precise mechanism, this selective block should throw further light on the physiology of transmission processes in this organ. Neither component corresponds to that described by Swedin (1971), who found that with long trains of 120–240 pulses each twitch was followed by a slow contraction which was abolished by α-adrenoceptor blocking drugs and by previous reserpiniza-

tion of the animals. The LSD-susceptible and the LSD-resistant components found in the present investigation are both unaffected by phenoxybenzamine and by reserpinization. For a more detailed discussion of Swedin's (1971) results the reader is referred to an earlier paper (Ambache *et al.* 1972*b*).

As to the site of action of LSD, the results are at present open to several interpretations. If we assume the operation, in the longitudinal muscle coat of the guinea-pig vas deferens, of only a single non-adrenergic motor transmitter then a presynaptic mechanism for the action of LSD would seem likely. For, if the LSD inhibition were due to a specific post-synaptic blockade of the motor transmitter at its receptors in the smooth muscle, the effect of LSD should not have ceased to be dose-dependent as the concentration of LSD was raised from 10^{-6} to 10^{-5} g/ml.; and the antagonism, already manifest at 10^{-9} g/ml. with short trains, should not have spared the responses elicited by longer trains, at the final concentration of 10^{-5} g/ml. Another observation which would be inconsistent with a post-synaptic receptor-blocking action of LSD was the fact that the degree of LSD inhibition was influenced by the frequency of stimulation. In all our experiments on the guinea-pig vas the responses to 5-pulse trains were larger at 10 Hz than at 40 Hz before LSD, but this difference was obliterated or reversed on exposure to LSD. The bigger initial response to 5-pulse trains at 10 Hz would indicate the availability to the muscle receptors of a larger quantity of the motor transmitter than at 40 Hz; if the LSD were acting post-synaptically at the receptors, there is no reason why it should have antagonized preferentially the larger amount of transmitter released by stimulation at 10 Hz. Although at the moment the balance of evidence would seem to point to a presynaptic site of action for LSD, this question cannot be settled until the identity of the motor transmitter in the vas is established.

On a presynaptic theory two possibilities come to mind. The first is that the effect of LSD might be due to a partial lowering of transmitter release at all train lengths. This could well result in the greater degree of inhibition observed with short than with long trains. With short trains, the total transmitter output is so low that, when reduced by LSD, it could easily fall below the threshold level required for receptor excitation. On the other hand, with long trains there is good evidence for an excessive output of transmitter, because the tension response elicited by 20 pulses at 10 Hz is usually no greater than that elicited by 10 or 15 pulses. The paralytic effect of the reduction in transmitter output by LSD would therefore be cushioned to a considerable extent by the transmitter excess; this would result in a lower degree of inhibition than is recorded with short trains. Such a hypothesis could provide a satisfactory explanation for the finding,

illustrated in Figs. 2 and 7, that LSD-inhibition was greater at 10 Hz than at 40 Hz with short trains but that with long trains it was greater at 40 Hz: if we assume the decay of transmitter to be greater the longer the interval between pulses, then clearly the shortening of the pulse intervals at 40 Hz could counterbalance the postulated decrease in transmitter output produced by LSD; and the effect would be more marked at liminal than at excess output-levels.

The second possibility would assume the existence of two stages, or processes, in the mechanism of the motor transmission: (1) an 'early', LSD-susceptible process responsible for the transmission of the first 4 or 5 impulses in a volley; and (2) a delayed, LSD-resistant process, coming into play after the first few impulses. It is premature to speculate about the nature of the presynaptic process predominant at short train lengths, which would be affected by this inhibitory action of LSD.

There appear to be certain resemblances in structural specificity between the inhibitory action of LSD on the motor transmission in the vas deferens and some of its actions on the C.N.S. As found in the vas deferens preparations in the present experiments, the psychotomimetic (hallucinogenic) action of LSD cannot be reproduced by methysergide or 2-bromo-LSD (Goodman & Gilman, 1965, pp. 206–207). In both situations, therefore, the activity is considerably reduced by N-methyl or 2-bromo substitution in the indole ring of the LSD molecule. Likewise, Holman, Shillito & Vogt (1971) observed that the sleep-inducing effect of clonidine in chicks could be prevented by LSD but not by methysergide. In the dihydroergotamine molecule, the hydrogenation of the double bond in the D ring, without any substitution in the indole ring, does not seem to result in any substantial reduction of the activity on the vas deferens.

The vas deferens preparation provides a convenient method for the study of the new type of activity of LSD. Further experiments on this isolated preparation may give greater insight into the mechanism of the central actions of LSD. Arising from the present finding with phentolamine on vasa, it would be of interest to know whether this drug could be used as an antidote to the hallucinogenic effect of LSD. For it is conceivable that in some C.N.S. synapses a transmission process is present which is analogous to that detected in the vas deferens with short trains, and that it is a synaptic inhibition by LSD of the same kind as has now been demonstrated in the vas, which is responsible for the hallucinogenic action on the CNS.

We are indebted to Dr Marthe Vogt and Dr G. C. R. Morris for advice and criticism in the preparation of the manuscript; and to Messrs P. Miall, P. Richardson and P. D. Smith for valuable assistance.

REFERENCES

AMBACHE, N. & ZAR, M. ABOO (1971). Evidence against adrenergic motor transmission in the guinea-pig vas deferens. *J. Physiol.* **216**, 359–389.

AMBACHE, N., DUNK, LINDA P., MIALL, P. & ZAR, M. ABOO (1971). Unexplained inhibitory action of D-lysergic acid diethylamide (LSD) on postganglionic motor transmission in the guinea-pig vas deferens. *Br. J. Pharmac.* **42**, 659–660*P*.

AMBACHE, N., DUNK, LINDA P., VERNEY, J. & ZAR, M. ABOO (1972*a*). Inhibitory nature of the adrenergic innervation in the guinea-pig vas deferens. *Br. J. Pharmac.* **44**, 359–360*P*.

AMBACHE, N., DUNK, LINDA P., VERNEY, J. & ZAR, M. ABOO (1972*b*). Inhibition of post-ganglionic motor transmission in vas deferens by indirectly acting sympathomimetic drugs. *J. Physiol.* **227**, 433–456.

BIRMINGHAM, A. T. & WILSON, A. B. (1963). Preganglionic and postganglionic stimulation of the guinea-pig isolated vas deferens preparation. *Br. J. Pharmac.* **21**, 569–580.

BOYD, HELEN, CHANG, V. & RAND, M. J. (1960). The anticholinesterase activity of some antiadrenaline agents. *Br. J. Pharmac.* **15**, 525–531.

GOODMAN, L. S. & GILMAN, A. (1965). *The Pharmacological Basis of Therapeutics*, 3rd edn., pp. 206–207. New York: Macmillan.

GYERMEK, L. (1961). 5-Hydoxytryptamine antagonists. *Pharmac. Rev.* **13**, 399–439.

HOLMAN, R. B., SHILLITO, ELIZABETH E. & VOGT, MARTHE (1971). Sleep produced by clonidine (2-(2,6-dichlorophenylamino)-2-imidazoline hydrochloride). *Br. J. Pharmac.* **43**, 685–695.

LARGE, B. J. (1965). Sympathetic β-receptors and the guinea-pig vas deferens. *Br. J. Pharmac.* **24**, 194–204.

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

SWEDIN, G. (1971). Studies on neurotransmission mechanisms in the rat and guinea-pig vas deferens. *Acta physiol. scand.* **83**, suppl. 369.

WAKADE, A. R. & KRUSZ, J. (1972). Effect of reserpine, phenoxybenzamine and cocaine on neuromuscular transmission in the vas deferens of the guinea-pig. *J. Pharmac. exp. Ther.* **181**, 310–317.