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EFFECTS OF LYSERGIC ACID DIETHYLAMIDE ON AUTONOMIC POST-GANGLIONIC TRANSMISSION

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SUMMARY

1. Six sites of autonomic post-ganglionic transmission were examined for susceptibility to LSD. Inhibition of transmission by LSD was confined to the three sympathetic junctions.

2. Inhibition of sympathetic transmission was maximal with short trains of pulses and declined considerably as train length was increased.

3. Evidence for a presynaptic mode of action was obtained. This was the predominant effect of LSD in the rat anococcygeus and dog retractor penis because α-adrenoceptor-blocking properties were feeble or absent; but in dog splenic strips LSD produced marked post-synaptic α-blockade.

4. The presynaptic inhibitory effect of LSD was unrelated to its 5-hydroxytryptamine-blocking property because it was not shared by methysergide. Neither was it mediated by prostaglandin release because it was unaltered by indomethacin, which suppresses prostaglandin synthesis.

5. In the rat anococcygeus and dog retractor penis larger doses of LSD induced slow contractions and, as a result of the concurrent block of motor transmission, revealed relaxation responses on transmural stimulation, caused by the excitation of sacral inhibitory fibres present in these muscles.

6. The LSD contractions were due to stimulation of post-synaptic α-adrenoceptors because they were abolished by phentolamine or yohimbine but were present as usual in preparations taken from reserpinized animals.

7. LSD blocked presynaptic α-adrenoceptors in the cholinergic motor fibres to the longitudinal muscle of the guinea-pig ileum.

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INTRODUCTION

An inhibition of sympathetic post-ganglionic motor transmission in the guinea-pig vas deferens by lysergic acid diethylamide (LSD) has been reported from this laboratory (Ambache, Dunk, Verney & Zar, 1973). Several observations suggested that the site of action was presynaptic, but this could not be fully proven because of uncertainties regarding the nature of the sympathetic motor transmitter to the longitudinal muscle in the vas deferens (Ambache, Dunk, Verney & Zar, 1972). However, the resemblance of the LSD inhibition to the presynaptic inhibition produced by noradrenaline was noted; and the fact that the LSD inhibition was antagonized by phentolamine suggested that it was, likewise, due to stimulation of inhibitory presynaptic adrenoceptors. Further investigations have now been carried out to determine how widespread is the susceptibility to LSD-inhibition at other autonomic post-ganglionic junctions. Six junctional sites have been examined, three of them sympathetic (with phentolamine-susceptible, and therefore undoubtedly adrenergic, motor transmission) and the other three, parasympathetic; the inhibition of post-ganglionic transmission was confined to the sympathetic junctions. The presynaptic nature of the LSD-inhibition could be clearly demonstrated in the rat anococcygeus and dog retractor penis preparations because it was not masked by any concurrent post-synaptic blockade of α-adrenoceptors; but in dog splenic strips LSD exerted a marked blocking action on post-synaptic α-adrenoceptors. LSD also blocked the presynaptic α-adrenoceptors in the cholinergic motor fibres to the longitudinal muscle of the guinea-pig ileum.

A preliminary account of some of these results has appeared previously (Ambache, Killick, Srinivasan & Zar, 1973).

METHODS

The isolated preparations described below were carefully desheathed and suspended, each in a 2 ml. organ bath at 35° C, containing Krebs–Henseleit solution of the following composition (mM): NaCl, 112·9; KCl, 4·69; $CaCl_2 . 2H_2O$, 2·52; $MgSO_4 . 7H_2O$, 1·5; KH_2PO_4, 1·18; $NaHCO_3$, 25·0; glucose, 22·2. Baths and reservoirs were bubbled with a 95 % O_2–5 % CO_2 mixture. Electrical stimulation was delivered intermittently via two parallel, vertical, built-in platinum electrodes, and the muscular contractions or relaxations were recorded isometrically by means of transducers. Details of the stimulating and recording apparatus have been given elsewhere (Ambache, Dunk, Verney & Zar, 1973). A suitable pulse width in the range 0·2–1 msec was selected for each type of preparation, restricting the excitation to nervous elements, as confirmed by the susceptibility of responses to tetrodotoxin. The frequency was fixed at 10 Hz in all but the few experiments mentioned on pp. 574–575; voltage was kept constant throughout each experiment. In order to avoid fatigue effects, seen in some tissues, train length was not allowed to exceed 30 pulses (20 for retractor penis).

The preparations were also made to contract with various spasmogens, before and after exposure to LSD. A constant procedure for the administration, timing of the exposure, and duration of wash was adopted in each case.

Rat anococcygeus. Adult, male Wistar rats weighing 200–450 g were killed by concussion and the paired anococcygeus muscles were excised individually by Gillespie's (1972) method. The experiments were conducted at a resting tension of *ca.* 0·1 g, achieved by setting up the preparation at an initial tension of 0·2 g. The pulse width was 1 msec in all these experiments.

Dog tissues. The spleen and retractor penis were excised soon after death from dogs of various breeds, killed by injecting an overdose of Nembutal.

(*a*) *Retractor penis.* For an illustration of the anatomy of this muscle in the dog see Langley & Anderson (1895*b*; Fig. 1). The scrotal sac was opened in the mid line and each testicle was retracted laterally. Connective tissue was dissected away to expose the retractor as a pale band of smooth muscle at the root of the penis, where it begins to be invaded by pink striated muscle fibres. In order to avoid inclusion of these skeletal fibres, the muscular band was divided at least 1 cm distal to the pink portion. It was then separated from the penis up to the prepuce, where it was cut from its attachment. The paired nature of this muscle becomes more apparent after it is desheathed. Some four to six preparations could be obtained from a single dog by cutting thin longitudinal strips, 3–4 cm in length, from each half.

The preparations were suspended at an initial tension of 0·5 g. The pulse width was fixed at 0·5 msec. A few preparations in which, for reasons unknown, motor transmission was not well maintained, were discarded.

(*b*) *Dog splenic capsule strips.* The peritoneal covering was removed from the excised spleen shortly after death and fine strips of the capsule, 3–4 cm long, 1–1·5 mm wide and 1 mm thick, were cut in the long axis of the organ.

Initial tension was 0·5 g and the pulse width usually 0·2 msec, but 0·5 msec in a few experiments.

Rabbit rectococcygeus. Adult, male, New Zealand White rabbits weighing 2–4 kg were killed by concussion and twin hemi-rectococcygeus preparations were made, as described previously (Ambache, Killick & Zar, 1973, 1974). The preparations were suspended at an initial tension of 0·5 g. The pulse width was 0·2 msec in all experiments.

Plexus-containing longitudinal muscle of guinea-pig ileum. Large albino guinea-pigs of the Dunkin–Hartley strain were killed by concussion and segments of the ileum were taken well above the ileocaecal valve and also from the terminal portion of the ileum (last 5–10 cm). Plexus-containing preparations were made from both regions, by the technique described in Ambache & Freeman (1968). Initial resting tension was 0·4 g and the pulse width was 0·2 msec in all experiments.

Reserpinization. The rats received two injections of reserpine phosphate solution (5 mg/ml. in distilled water), in a daily dose of 10 mg/kg S.C. on the first day, I.P. on the second, and were killed on the third day. This high dose of reserpine was necessary to achieve a complete block of motor responses to electrical stimulation in the anococcygeus muscle.

Indomethacin treatment. Rats received an I.P. injection of indomethacin, 20 mg/kg, 2 hr before sacrifice. The solution of indomethacin was prepared immediately before each injection, by dissolving the required amount of the drug in a minimal quantity of 99% ethanol (*ca.* 1/60 w/v), and then diluting with Krebs–Henseleit solution to give a final concentration of 1%. The anococcygeus muscles excised from these treated animals were washed for 1 hr in a slow stream of Krebs–Henseleit solution at 35° C, in order to remove any prostaglandins released by the trauma of excision.

Drugs. Except for indomethacin (Merck, Sharp and Dohme), prostaglandin E_2 (PGE_2; Upjohn) and tetrodotoxin (Sankyo), all dosages refer to the respective salts, namely: acetylcholine chloride (Hopkin & Williams), atropine sulphate (Hopkin & Williams), D-2-bromolysergic acid diethylamide hydrogen tartrate (BOL 148; Sandoz), guanethidine sulphate (Ciba), 5-hydroxytryptamine creatinine sulphate (Sigma), D-lysergic acid diethylamide tartrate (LSD-25; Sandoz: effective mol. wt. taken as consisting of 1 mole of LSD + ½ mole of tartaric acid), methysergide hydrogen maleate (Sandoz), L-noradrenaline hydrogen tartrate (Koch-Light), phentolamine methane sulphonate (Ciba), propranolol hydrochloride (I.C.I.), reserpine phosphate (Ciba), *p*-tyramine hydrochloride (Sigma) and yohimbine hydrochloride (Sigma).

Tests of statistical significance (e.g. Fig. 1) were made by comparisons of correlated means and application of Student's *t* test; a level of significance of $P < 0.05$ was taken.

RESULTS

Effects of LSD on sympathetic post-ganglionic transmission

Inhibition of motor transmission in rat anococcygeus

Field stimulation of anococcygeus preparations elicited twitch responses which grew in height as train length was increased (Fig. 1). Train lengths not exceeding 30 pulses were used, with the frequency fixed usually at 10 Hz and pulse width at 1 msec. At this pulse width the twitch responses were entirely neurogenic in origin, because they were completely or virtually abolished (Fig. 1, left panel, bottom curve) by tetrodotoxin, given in concentrations which did not affect contractions elicited by noradrenaline or by acetylcholine. The twitches were also virtually extinguished by phentolamine (Fig. 1, right panel, bottom curve), which confirms the adrenergic nature of the motor transmission in this muscle.

In sixteen experiments exposure of the muscle to LSD, 25 nM–1·25 μM ($0{\cdot}1–5 \times 10^{-7}$ g/ml.), for 5 or more min markedly reduced the twitch responses to short trains, e.g. 1–5 pulses; but the degree of inhibition caused by LSD declined progressively as the train length was increased. The inhibitory effect of LSD was fairly long-lasting; recovery was slow after the drug was washed out and could take up to 2 hr. These experiments showed that a concentration of 125 nM LSD regularly produced marked inhibition of motor transmission, without the tonic effect obtained at higher concentrations (see below). Fig. 1 illustrates five further experiments in which the concentration of LSD was fixed at 125 nM to avoid such tonic effects. Each experiment was performed on twin anococcygeus preparations taken from the same animal. The inhibitory effect of LSD was statistically significant up to 20 pulses in all the preparations and was, again, much more marked with short than with long trains.

In a few experiments the train length was fixed at 15 or 30 pulses but the frequency of stimulation was varied between 0·25 and 50 Hz. With these

long trains, the inhibitions produced by LSD, 0·25 μM, were small at 2–50 Hz, but became very pronounced at lower frequencies (0·25–1 Hz); for example, with 30 pulses there was only 9% inhibition at 50 Hz, but 94% at 0·25 Hz.

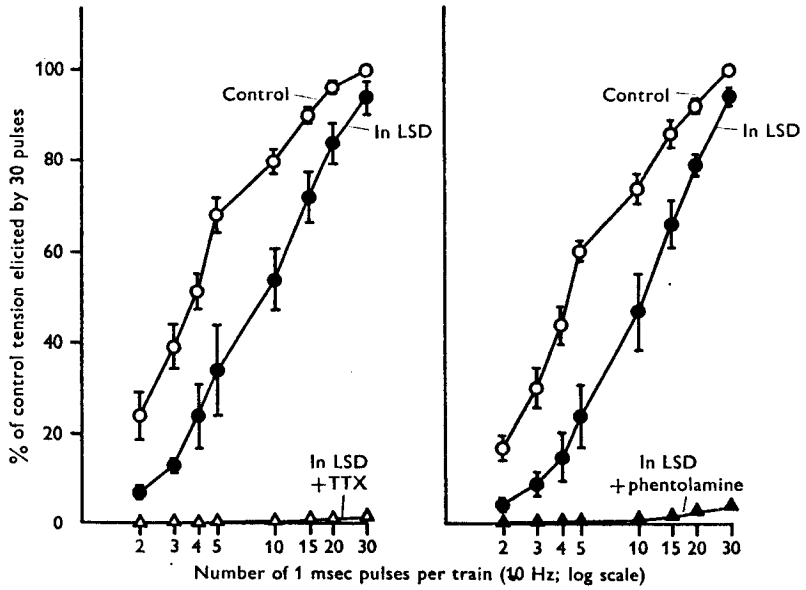

Fig. 1. Rat anococcygeus preparations from five different animals. Influence of train length on inhibition of post-ganglionic motor transmission by LSD. In each experiment twin preparations were taken from the same animal. The ten muscles were stimulated transmurally at 1 min intervals by 2–30 pulses (1 msec; 10 Hz) of constant voltage; the responses at each train length have been expressed as percentages of the control tension elicited by 30 pulses. The left and right curves, each representing five muscles, give the means of these responses; S.E. of means are shown by vertical bars. ○—○, before LSD; ●—●, in the presence of LSD, 125 nM (5×10^{-8} g/ml.), given 10 min previously. The LSD-resistant component was almost entirely neurogenic because it was virtually abolished, subsequently, by tetrodotoxin, 0·31 μM (10^{-7} g/ml.), in five of the preparations (left side, △—△) and by phenotolamine, 2·6 μM (10^{-6} g/ml.), in the other five (right side, ▲—▲), showing that it is adrenergic.

Left curves: $P < 0{\cdot}01$ for 3, 4 and 20 pulses; $P < 0{\cdot}02$ for 2, 5, 10 and 15 pulses and $P > 0{\cdot}1$ for 30 pulses.

Right curves: $P < 0{\cdot}01$ for all points except for $P < 0{\cdot}05$ at 10 and 30 pulses.

The inhibition by LSD of twitch responses to single pulses or to short bursts was due to a presynaptic action because it was not associated with a comparable reduction in the response to administered noradrenaline. In fourteen out of twenty-one trials on preparations from eleven animals

a result of the type illustrated in Fig. 2 was obtained, i.e. the twitch-inhibition by LSD (25 nM–1·25 μM) was in fact associated with an augmentation in noradrenaline responses of 5–109 % (mean 26 %, S.E. of mean ± 8). In the experiment of Fig. 2 the degree of potentiation increased

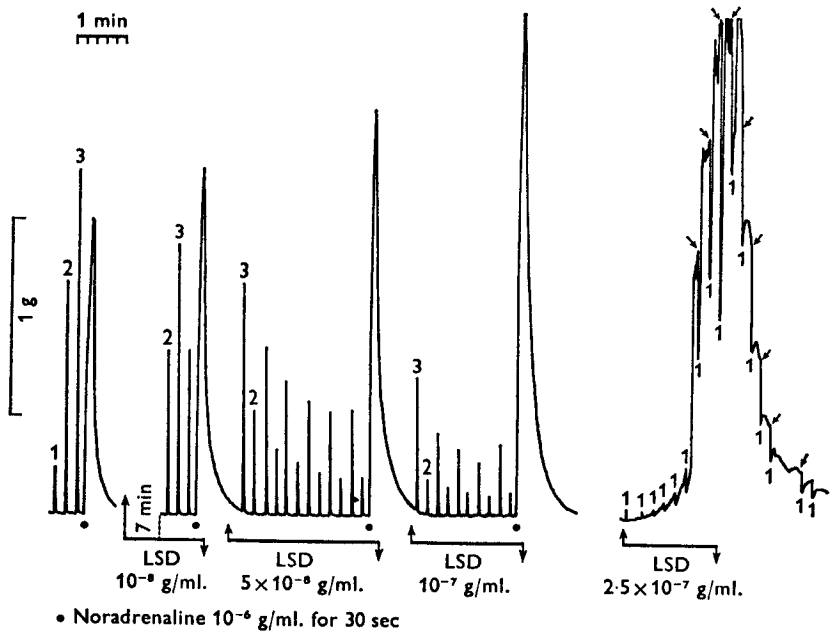

Fig. 2. Rat anococcygeus. Presynaptic site of action for the LSD inhibition. Twitches elicited at 1 min intervals by 1, 2 or 3 pulses (1 msec; 10 Hz), as indicated by superscripts or subscripts. At the dots, exposure for 30 sec to noradrenaline, 2·96 μM (10^{-6} g/ml.). Between the arrows LSD, 25, 125, 250 and finally 625 nM (10^{-8}, 5×10^{-8}, 10^{-7} and $2{\cdot}5\times10^{-7}$ g/ml.). Note that LSD inhibition of twitch responses is greater for 2 than for 3 pulses, and occurs without reduction in noradrenaline contractions, which are in fact potentiated. The last panel shows the delayed contraction produced by the highest concentration of LSD and the reversal (at the oblique arrows) of the response to single pulses, revealing the effect of the inhibitory nerves. Further explanations in text.

as the dose of LSD was raised. In the remaining seven trials LSD, 125–250 nM, produced some reduction in noradrenaline responses (3–53 %; mean 16 %, S.E. of mean ± 7); this reduction was, however, much less marked than the inhibition of motor transmission (e.g. with 4 pulses: 37–85 %; mean 54 %, S.E. of mean ± 6). Selecting from these experiments those in which the dose of LSD was fixed at 125 nM, for the reasons already given above, the following result was obtained: in thirteen preparations this

concentration of LSD potentiated the contractions elicited by noradrenaline, 2·96 or 5·92 μM, by a mean of 8·4% (S.E. of mean ± 7·4), which was not statistically significant ($P > 0.1$).

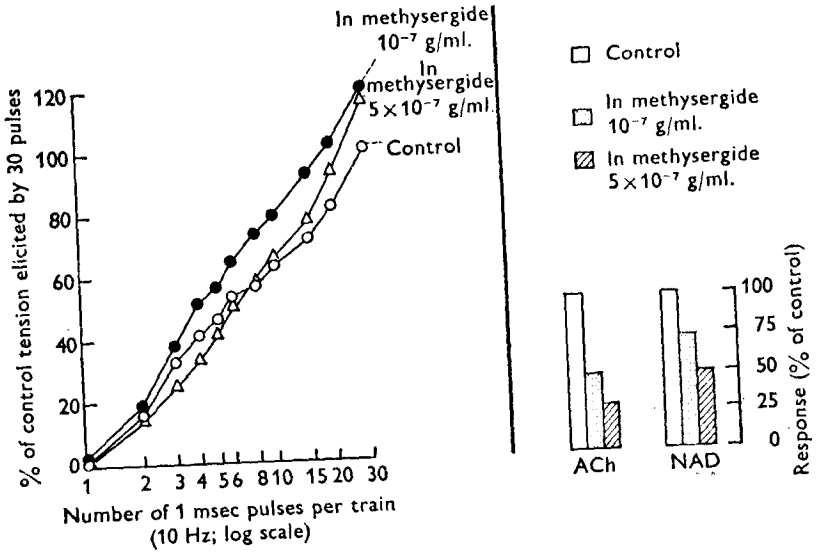

Fig. 3. Rat anococcygeus. The inhibitory effect of LSD is not reproduced by methysergide. *Left panel*: motor responses to transmural stimulation at 1 min intervals with 1–30 pulses (1 msec; 10 Hz). All responses have been expressed as percentages of the control tension elicited by 30 pulses. ○—○, before methysergide; ●—●, in methysergide, 0·21 μM (10^{-7} g/ml.); △—△, in methysergide, 1·06 μM (5×10^{-7} g/ml.). Note the virtual absence of inhibition with methysergide and its slight potentiating effect. *Right panel*: same preparation. Responses to fixed doses of acetylcholine, 27·5 μM (5×10^{-6} g/ml.) or of noradrenaline, 2·96 μM (10^{-6} g/ml.) before and during exposure to methysergide, 0·21 or 1·06 μM (1 or 5×10^{-7} g/ml.). Methysergide depressed the acetylcholine and noradrenaline responses, which are expressed as percentages of their respective controls.

The inhibition of motor transmission by LSD was unrelated to its well known ability to antagonize 5-hydroxytryptamine (5-HT). Thus, when given in doses which inhibited 5-HT contractions, methysergide, which is an even more potent antagonist of 5-HT than LSD, was at least 45 times weaker, on a molar basis, than LSD in inhibiting motor transmission. As shown in Fig. 3, methysergide, 0·21–1·1 μM, caused a dose-dependent depression of muscle responses to administered acetylcholine or noradrenaline, but did not block the motor transmission. With 0·21 μM, the transmission was slightly potentiated at all train lengths; the increase in methysergide concentration to 1·1 μM reduced this potentiation.

The results obtained with another 5-HT antagonist, BOL 148 were not clear cut, because it had weak α-adrenoceptor blocking properties, shown by the fact that it potentiated acetylcholine, but depressed noradrenaline-contractions. With 0·18 μM, the motor transmission was slightly inhibited, but with 0·9 μM it was slightly potentiated at all train lengths.

It has been suggested by Hedqvist (1970) that endogenous prostaglandins may regulate the release of noradrenaline from sympathetic nerves by a negative feed-back mechanism. In our experiments PGE_2 (14·2–142 nM), whilst potentiating contractions elicited by noradrenaline or acetylcholine, depressed the electrically induced twitch responses. The possibility that the inhibitory effect of LSD was mediated through a release of prostaglandin was therefore examined on anococcygeus preparations taken from rats which had been pre-treated with indomethacin, a drug known to suppress prostaglandin synthesis (Vane, 1971). The inhibition caused by LSD was found to be of the same order in muscles from treated as in those from untreated animals.

Muscle-contracting effect of LSD in higher concentrations. When, as in the experiment of Fig. 2 (last panel), the concentration of LSD was increased beyond the level at which it had produced presynaptic inhibition only, it began to exert an overt muscle-contracting action. The last panel in Fig. 2 also shows that during the rise in muscle tone produced by the highest concentration of LSD the residual motor responses to single pulses, already depressed by the preceding LSD treatments, were gradually replaced by relaxation responses which were most marked at the peak of the LSD contractions. These results are in agreement with Gillespie's (1972) report that the rat anococcygeus muscle possesses, in addition to the adrenergic motor innervation, an inhibitory innervation, the relaxant effect of which is revealed, on transmural stimulation, only if the motor transmission has been blocked and if some tone has been induced in the muscle.

The muscle-contracting effect of LSD was antagonized by α-adrenoceptor blocking agents. Thus, LSD failed to produce contractions in preparations pre-treated with phentolamine, 2·6 μM, or yohimbine, 25·6 μM; and, in muscles already contracted by LSD, the addition of phentolamine at the height of the contraction caused an immediate relaxation (Fig. 4).

It is known that both phentolamine and yohimbine can block not only adrenoceptors but also 5-HT receptors (Åström & Samelius, 1957). But the LSD contraction in the rat anococcygeus does not involve such receptors, because it was unaffected by the potent 5-HT blocker, methysergide, 2·1 μM.

In order to determine whether the adrenoceptors concerned in the LSD-contraction were located in the smooth muscle or at the adrenergic nerve endings, the effect of LSD was further examined in preparations taken from

rats pre-treated with reserpine. Fig. 5 illustrates such an experiment, conducted in parallel on two preparations, one of which was taken from a reserpinized and the other from a normal rat. The following criteria for successful depletion of endogenous noradrenaline in the 'reserpinized' preparations were satisfied: (1) paralysis of adrenergic motor transmission (cf. *A'* with *A* in Fig. 5) and (2) loss of the contractile response to noradrenaline-releasing drugs, such as tyramine or guanethidine (cf. *B'* and *C'* with *B* and *C*). LSD never failed to contract the reserpinized preparations (cf. *D'* with *D* in Fig. 5), which showed that the LSD contraction was due to a direct action on the smooth muscle.

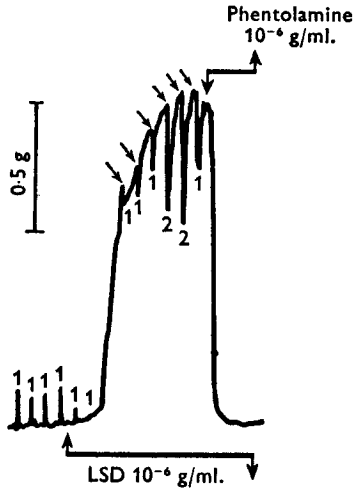

Fig. 4. Rat anococcygeus. Antagonism of the LSD contraction by phentolamine. Preparation stimulated at 1 min intervals by 1 or 2 pulses (1 msec; 10 Hz), as indicated by superscripts or subscripts. The introduction of LSD induces a delayed, slow contraction, which is promptly abolished by phentolamine. Note, also, that the size of the inhibitory responses (oblique arrows) revealed by LSD is related to the tone of the preparation.

Inhibition of motor transmission in dog retractor penis

Anatomically this muscle is a continuation of the caudo-anal muscle (Langley & Anderson, 1895*b*), which is the homologue of the rat anococcygeus muscle. The retractor penis is innervated by two distinct sets of post-ganglionic fibres: (1) adrenergic motor fibres and (2) parasympathetic, inhibitory fibres, the neurotransmitter of which is still unknown (Luduena & Grigas, 1966).

The contractions elicited by field stimulation of this preparation with 0·5 msec pulses were neurogenic because they were abolished by

tetrodotoxin, 0·6 μM, or by phentolamine, 2·6 μM. As in the rat anococcygeus, LSD, 25–125 nM, produced an inhibition of motor responses to electrical stimulation, which was more marked with short trains (Fig. 6*B*).

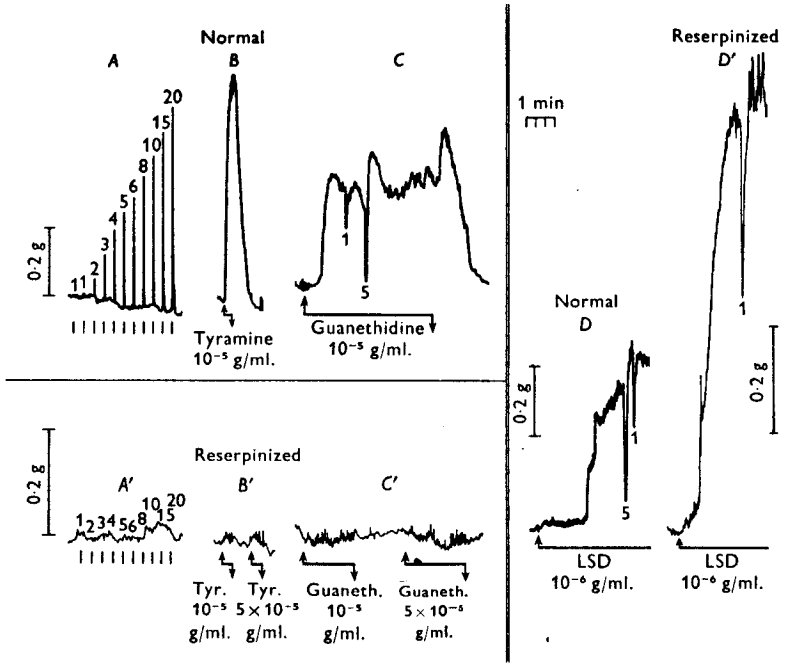

Fig. 5. Rat anococcygeus. Reserpinization does not abolish the LSD contraction. Parallel experiments on a control, normal preparation (*A*–*D*) and on a preparation taken from a reserpinized rat (*A'*–*D'*). *A* and *A'*: effect of transmural stimulation at 1 min intervals with 1–20 pulses (1 msec; 10 Hz; number of pulses indicated by superscripts or subscripts). Note total motor paralysis in the 'reserpinized' preparation. *B* and *B'*: tyramine contracts the normal but not the 'reserpinized' preparation. *C* and *C'*: guanethidine contracts the normal but not the 'reserpinized' preparation. *D* and *D'*: LSD contracts both preparations. Note also the appearance of inhibitory responses in *C*, *D* and *D'*.

In addition, LSD had a direct action on the muscle fibres, which resulted in delayed relaxation after electrically induced contractions or, at high LSD concentrations (1·25–5·0 μM), in overt spasms. With intermediate concentrations of LSD, 0·125–0·50 μM, this action led to the appearance of multiphasic responses to transmural stimulation and to a potentiation of noradrenaline contractions (Fig. 6, upper record). Because of these contractile or irritant effects of LSD, it was sometimes necessary, in order to detect the presynaptic action of LSD, to interrupt the electrical stimulation during the presence of LSD in the organ bath and for a further 3–15 min

after the LSD was washed out, e.g. in the experiment of Fig. 6, upper record.

The inhibition of motor transmission by LSD could again be attributed to a presynaptic action. Thus, in two out of six preparations the inhibitions recorded during the presence of, or after previous treatment with, LSD were associated with an augmentation of noradrenaline contractions by 15–40%. In the remaining 4 preparations LSD reduced the height of noradrenaline contractions by 9–26%, a result suggestive of a slight, concurrent α-blockade of post-synaptic adrenoceptors. But the predominantly presynaptic effect of LSD was evident from the disproportionately greater inhibition of motor transmission, e.g. Fig. 6, lower record.

The contracted state of the preparations produced by higher concentrations of LSD, 1·25–5·0 μM, combined with the presynaptic inhibition of the motor nerve fibres, led to a reversal of responses induced by 1 or 2 pulses, revealing the effect of the inhibitory innervation.

The LSD-contraction was prevented or abolished by α-adrenoceptor blocking agents, in parallel with noradrenaline- or tyramine-contractions.

Dog splenic strips: additional α-adrenoceptor blocking action of LSD

Field stimulation with trains of 2–20 pulses of 0·2 or 0·5 msec elicited graded contractions, which were spaced at 90 sec intervals because relaxation was slow. Contractions could also be elicited by noradrenaline, 3 μM, or by tyramine, 11·6–58·0 μM. The contractions elicited by field stimulation were neurogenic because they were abolished by tetrodotoxin, 0·6 μM, or by phentolamine, 5·3 μM; they were unaffected by atropine, 0·29 μM.

Inhibition of motor transmission by LSD, 0·025–1·25 μM, was more marked with short trains of pulses (Fig. 7*B*). In two out of six experiments the inhibition of neurogenic twitches was not associated with a reduction in the height of noradrenaline contractions (Fig. 7*B*), revealing the presynaptic action of LSD already noted on the other sympathetic preparations. But in the remaining four experiments any possible presynaptic effect was masked by the fact that LSD exerted a marked blocking action on post-synaptic α-adrenoceptors, shown by the great reduction in noradrenaline contractions (Fig. 7, last panel).

The dog splenic strips differed from the rat anococcygeus and dog retractor penis preparations in that high concentrations of LSD failed to elicit outright contractions, producing at most a slight increase in tone in only two of the experiments. Thus, as far as the post-synaptic α-adrenoceptors of the splenic capsule are concerned, the antagonist properties of LSD outweigh its agonist properties.

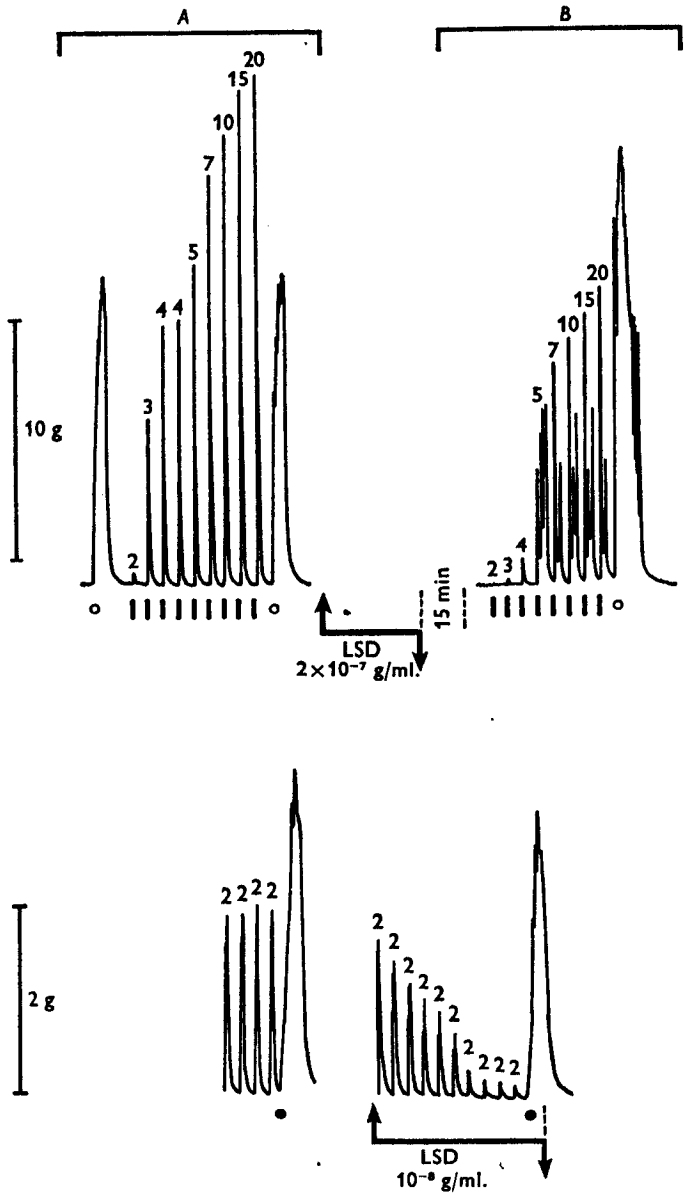

Fig. 6. For legend see facing page.

Failure of LSD to inhibit parasympathetic post-ganglionic transmission

Cholinergic motor transmission to the rabbit rectococcygeus. The rectococcygeus muscle receives an extrinsic parasympathetic motor innervation from the sacral nerves (Langley & Anderson, 1895*a*, 1896). A brief account of some of its pharmacological properties has been given by McKirdy (1972). The post-ganglionic transmission is cholinergic (Ambache, Killick & Zar, 1973, 1974).

As shown in Fig. 8, LSD produced a dose-dependent potentiation of the twitches elicited by 1–30 pulses (0·2 msec; 10 Hz) in hemi-rectococcygeus preparations; this effect was more marked with short than with long trains. Twitch-inhibition of the kind seen in the sympathetically innervated preparations was never recorded.

Noradrenaline inhibits the cholinergic motor transmission in this preparation (Ambache, Killick & Zar, 1974); LSD, 0·5 μM, did not antagonize this action of noradrenaline, which was obtained in the presence of the β-adrenoceptor blocking agent, propranolol, 6·8 μM.

Cholinergic motor transmission to the longitudinal muscle of the guinea-pig ileum. Sixteen experiments were carried out on plexus-containing preparations of the longitudinal muscle from the guinea-pig ileum. Fourteen of the preparations were taken well above the ileocaecal valve and two from the terminal portion of the ileum, which is known to differ from the rest of the gut in that excitatory α-adrenoceptors are present near the ileocaecal junction (Munro, 1951); no difference was found in the present experiments. Thus, in both types of preparation the twitches elicited by transmural stimulation with single pulses of 0·2 msec width, delivered at

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Legend to Fig. 6.

Fig. 6. Dog retractor penis. Presynaptic inhibition of post-ganglionic motor transmission in two contrasting preparations taken from different animals. Twitches were elicited by transmural stimulation at 1 min intervals with trains of 0·5 msec pulses delivered at 10 Hz (number of pulses indicated by superscripts); at ○ or ●, noradrenaline administered for 1 min during interruption of stimulation.

Upper preparation, panel *A*: responses to 2–20 pulses before LSD. Between Panels *A* and *B* the preparation was exposed for 11 min to LSD, 0·5 μM (2×10^{-7} g/ml.), which produced a large contraction (not shown). Panel *B*: responses recorded 15 min after the LSD was washed out. Note the inhibition of transmission despite potentiation of the response to noradrenaline, 2·96 μM (○, 10^{-6} g/ml.).

Lower preparation: decline in motor transmission during 11 min exposure to LSD, 25 nM (10^{-8} g/ml.). There is a 95% inhibition of responses to 2 pulses with only 9% reduction in the response to noradrenaline, 5·92 μM (●, 2×10^{-6} g/ml.).

1 min intervals, were largely due to a cholinergic motor component, because when atropine, 0·28–1·44 μM, was given, it produced a 75–100% reduction in twitch height (Fig. 9).

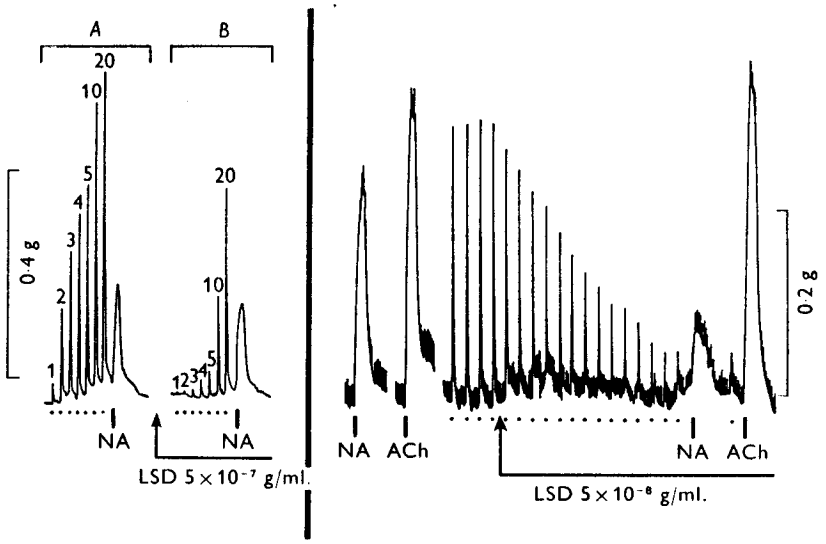

Fig. 7. Dog splenic capsule strips. Dual inhibitory action of LSD in two contrasting preparations obtained from different animals. Twitches elicited at 1 min intervals by 1–20 pulses of 0·2 msec width, delivered at 10 Hz (number of pulses indicated by superscripts). At 'NA', noradrenaline and at 'ACh', acetylcholine; 1 min contacts.

First preparation (panels A and B): the action of LSD, 1·25 μM (5×10^{-8} g/ml.) is mainly presynaptic: there is a greater inhibition of responses to short than to long trains, without comparable reduction in the response to noradrenaline, 5·92 μM (2×10^{-6} g/ml.).

Second preparation (last panel): at the dots, transmural stimulation with 3 pulses. Note the α-adrenoceptor blocking effect of LSD, 125 nM (5×10^{-8} g/ml.), reducing responses to noradrenaline, 2·96 μM (10^{-6} g/ml.), but potentiating those to acetylcholine, 1·1 μM (2×10^{-7} g/ml.), and masking any possible concurrent presynaptic action of LSD.

LSD failed to inhibit this cholinergic transmission, when given either in the low concentrations (25–125 nM) which had never failed to produce a presynaptic inhibition in the sympathetically innervated preparations, or in higher concentrations (0·25–10 μM).

In one of these experiments the plexus-containing preparation was suspended together with a rat anococcygeus muscle in the same organ bath and the two preparations were stimulated simultaneously with trains of 2 or 3 pulses at a pulse width of 0·2 msec. On administration of LSD, 0·5 μM, the twitches of the anococcygeus were promptly inhibited but those

of the plexus-containing intestinal preparation showed no decline; the same result was obtained at a pulse width of 1 msec. There was no inhibition of the twitches in the gut preparation even after a twentyfold increase in the concentration of LSD to 10 μM.

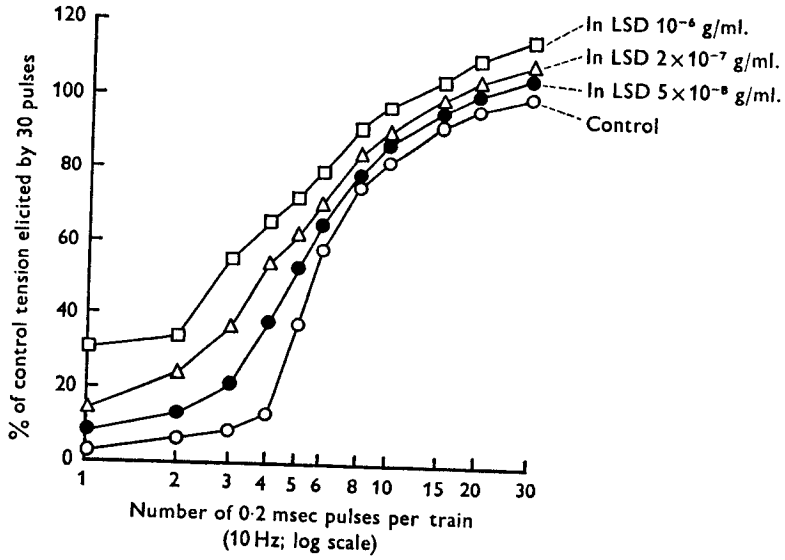

Fig. 8. Rabbit hemi-rectococcygeus preparation. The cholinergic post ganglionic motor transmission is not inhibited by LSD. Transmural stimulation at 1 min intervals with 1–30 pulses (0·2 msec; 10 Hz). Each twitch response has been expressed as a percentage of the control tension elicited by 30 pulses. ○—○, before LSD. ●—●, in LSD, 125 nM (5×10^{-8} g/ml.). △—△, in LSD, 0·5 μM (2×10^{-7} g/ml.). □—□, in LSD, 2·5 μM (10^{-6} g/ml.). Note progressive potentiation of all responses with increasing doses of LSD.

In most of the experiments LSD, especially in higher concentrations, produced a rise in tone, associated with small rhythmic contractions, and potentiated the motor responses to electrical stimulation and to administered acetylcholine (Fig. 9).

These results showed that LSD had failed to stimulate the inhibitory presynaptic α-adrenoceptors in the cholinergic motor endings of this preparation. On the other hand, LSD did produce a block of these presynaptic adrenoceptors (Fig. 9). In ten preparations, treated with propranolol, 6·8 μM, in order to abolish or reduce the contribution of β-adrenoceptors, powerful inhibition of motor transmission was recorded on administration of noradrenaline, 3–15 μM; this effect of noradrenaline was largely presynaptic because there was little or no reduction in acetylcholine contractions elicited during the noradrenaline-inhibition of motor

transmission (Fig. 9). The inhibition of motor transmission by noradrenaline was antagonized by LSD, 1·25–5 μM; at lower concentrations (0·5–1·25 μM) the LSD block of noradrenaline action was marked in some preparations but weak in others.

There was some evidence that LSD also produced some block of post-synaptic, α-adrenoceptors in the muscle because it antagonized the slight inhibitory reduction of acetylcholine contractions sometimes produced by noradrenaline in the presence of propranolol.

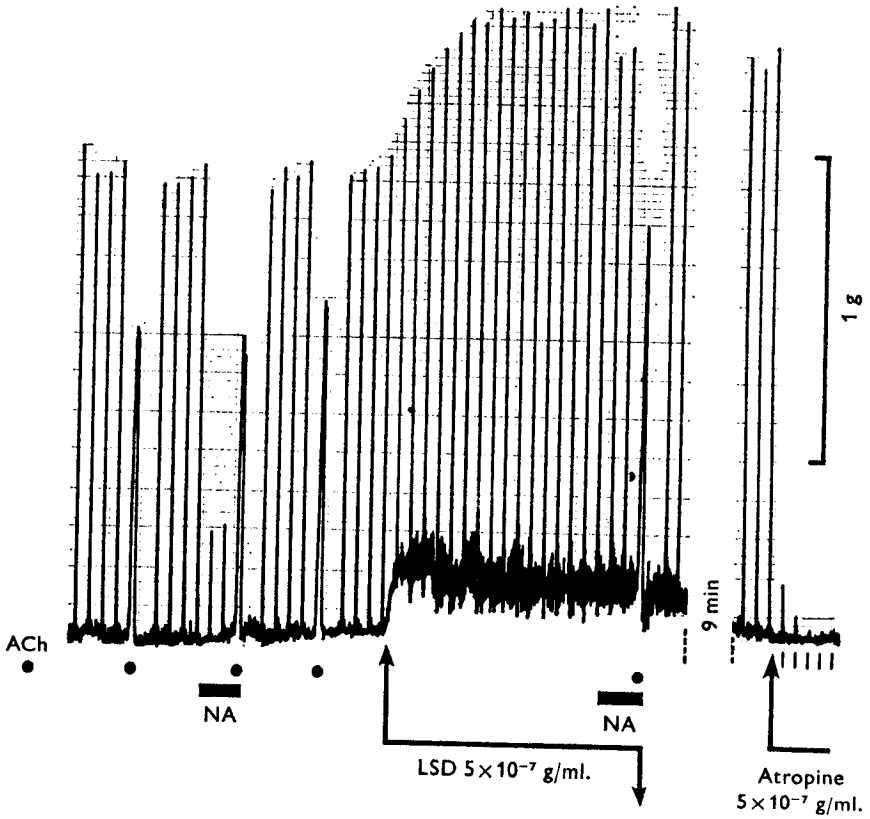

Fig. 9. Guinea-pig plexus-containing longitudinal muscle from ileum. LSD fails to inhibit the cholinergic post-ganglionic motor transmission but blocks its presynaptic inhibition by noradrenaline. Twitches were elicited at 1 min intervals by transmural stimulation with single 0·2 msec pulses; at the dots, acetylcholine, 0·11 μM (2×10^{-8} g/ml.), for 20 sec. Propranolol, 6·8 μM (2×10^{-6} g/ml.), present throughout. At the bars, noradrenaline, 2·96 μM (10^{-6} g/ml.), administered for 200 sec. Between the first and second arrows, LSD, 1·25 μM (5×10^{-7} g/ml.), antagonizes the inhibition of motor transmission by noradrenaline. At the end of the experiment, atropine, 1·44 μM (5×10^{-7} g/ml.), completely extinguishes the responses to transmural stimulation.

Non-cholinergic sacral inhibitory transmission to the rat anococcygeus. The inhibitory innervation of the anococcygeus appears to be autonomic in origin and to correspond to the parasympathetic innervation of the retractor penis (Langley & Anderson, 1895*b*; Luduena & Grigas, 1966). It has been traced back to spinal segments L_5–S_2 by Gillespie & McGrath (1973), who established its distinctness from the sympathetic motor innervation and, with hexamethonium, showed the presence of a ganglionic relay in this pathway. The post-ganglionic endings within the muscle itself differ from the motor endings in the rabbit rectococcygeus in that they are non-cholinergic (Gillespie, 1972; Gillespie & McGrath, 1973), although in both cases the nerve fibres concerned are of sacral origin.

The failure of LSD to depress this inhibitory transmission is illustrated in Fig. 10. The records show the inhibitory responses to 1 or 2 pulses before and during exposure to LSD, 0·25–1·25 μM, in an experiment in which guanethidine, 8 μM, was used to induce tone. Similar results were obtained in the experiments of Figs. 2, 4 and 5 (*D* and *D'*), in which LSD itself was used to contract the preparations, after which the inhibitory responses to electrical stimulation could be elicited.

Thus, LSD did not appear to affect adversely this non-cholinergic, autonomic transmission.

DISCUSSION

These results show that, besides its potentiating effects, LSD can exert four main types of effect at autonomic junctions: it can act pre- or post-synaptically, and either as an agonist or as an antagonist at adrenoceptors.

The three sympathetically innervated muscles which have been chosen for this investigation are known to be free from the anomalous resistance of the motor transmission to α-adrenoceptor blocking agents, which is found in the vas deferens of some species; in all three, the post-ganglionic motor transmitter is undoubtedly noradrenaline. This has made it possible to demonstrate the presynaptic nature of the inhibition that is produced by LSD, acting as an agonist, in nerve fibres which are classified as sympathetic. The investigation was prompted by similar findings, made earlier with LSD on guinea-pig and rat vasa (Ambache *et al.* 1973), where the hypogastric motor innervation is also classifiable, anatomically, as sympathetic. However, reasons have been given previously (Ambache & Zar, 1971; Ambache *et al.* 1972) for doubting whether the post-ganglionic motor transmission to the longitudinal muscle layer(s) in the vas is mediated by noradrenaline in those species in which it is phentolamine-resistant, such as the guinea-pig, rat and rabbit (but not always in the dog; N. Ambache & S. W. Killick, 1974, unpublished observations). The susceptibility to phentolamine of the motor transmission to the circular sheet

has never been examined. Confining ourselves to longitudinal muscle, however, the identity of the sympathetic motor transmitter becomes uncertain when phentolamine-resistance is present and when tyramine, which releases endogenous noradrenaline, fails to mimic the motor transmission. The pharmacological effects of LSD do not throw any further light upon this matter. Unlike phentolamine or tyramine, LSD

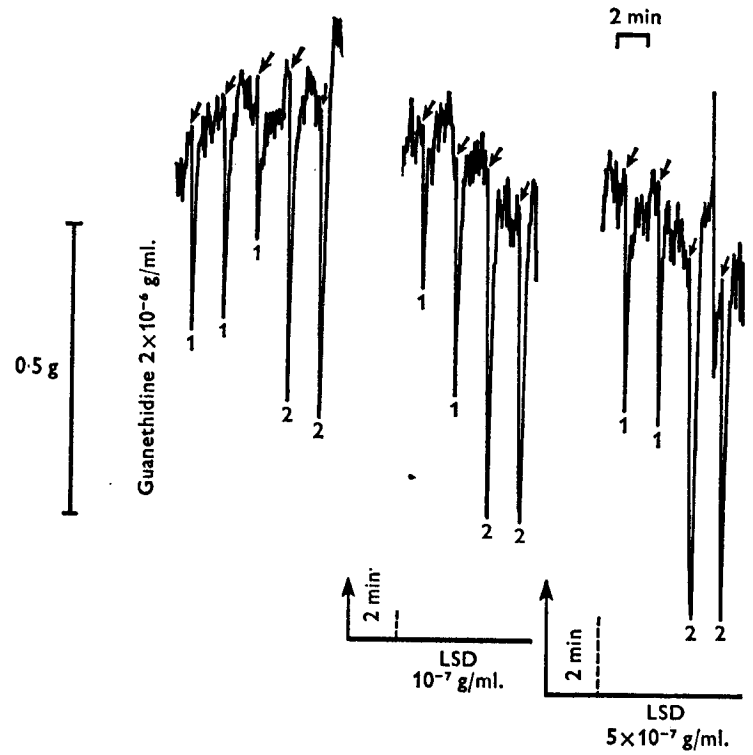

Fig. 10. Rat anococcygeus. LSD does not affect the post-ganglionic inhibitory transmission. Motor paralysis and tone were induced by guanethidine, 8·08 μM (2×10^{-6} g/ml.) present throughout the experiment. Note, at the oblique arrows, relaxations produced by 1 or 2 pulses (1 msec; 10 Hz; number of pulses indicated by subscripts) before (panel 1) and during (panels 2 and 3) exposure to LSD, 0·25 or 1·25 μM (1 or 5×10^{-7} g/ml.).

fails to distinguish between the phentolamine-resistant sympathetic motor junctions of the vas deferens and other sympathetic motor junctions, at which, as demonstrated again in some of the present experiments, phentolamine never fails to block post-ganglionic transmission, and tyramine to mimic it. The use of LSD merely shows that presynaptic adrenoceptors are present at both types of sympathetic junction, without indication as to the nature of their transmitter.

As to the mechanism underlying the presynaptic action of LSD, it is possible to rule out interference with impulse conduction or with ionic fluxes which are essential for transmitter-release, because LSD did not inhibit the parasympathetic transmissions. Mediation of the LSD-inhibition by prostaglandin release also appears unlikely from the findings after indomethacin treatment.

The present results and those obtained earlier on guinea-pig vasa (Ambache *et al.* 1973) suggest that the presynaptic inhibition by LSD is due to an agonist effect upon phentolamine-susceptible adrenoceptors located at sympathetic endings. Such a concept would link with Häggendal's (1970) hypothesis that endogenously released noradrenaline, acting on presynaptic α-adrenoceptors, can suppress the further release of noradrenaline; for supporting evidence, see Dubocovitch & Langer (1974). It seems possible that in sympathetic junctions of the phentolamine-susceptible type the LSD receptors are identical with the presynaptic α-adrenoceptors involved in feed-back inhibition, since both are blocked by phentolamine. This matter could not be put to the test on the three sympathetically innervated muscles used because, in all of them, phentolamine would block not only pre- but also post-ganglionic α-adrenoceptors, resulting in paralysis of the motor transmission under investigation. But the vas deferens is a useful preparation in this context because, as we have seen, the sympathetic motor transmission to the longitudinal muscle layer is not paralysed by α-adrenoceptor blockers in most species. This unique property made it possible to show previously on vasa (Ambache, Dunk, Verney & Zar, 1973) that the presynaptic inhibition produced by LSD was indeed abolished by phentolamine, like the inhibition of motor transmission by noradrenaline (also obtained with short trains of pulses; Ambache & Zar, 1971); on the other hand, phenoxybenzamine was less effective in preventing the inhibitions produced by noradrenaline or by LSD, showing that these receptors differed somewhat from classical α-adrenoceptors.

As noted before on guinea-pig vasa, the presynaptic inhibition produced by LSD was best seen with short trains of pulses, declined as train length was increased and became very small at 20–30 pulses. Further research is required to find out the underlying mechanism for this greater inhibition by LSD of motor responses to brief volleys. This is one of the characteristics of the presynaptic effect of LSD which distinguish it from the type of nerve-block which is produced by guanethidine at both kinds of sympathetic endings. It is well known that in vasa guanethidine is able to extinguish all motor responses to electrical stimulation, irrespective of train length; for example, the responses obtained with 375 pulses by Birmingham & Wilson (1963, Fig. 6). On the other hand, LSD spares the responses of vasa to long trains, even if given in a concentration 1000–

10,000 times greater than that which affects responses to short trains (Ambache, Dunk, Verney & Zar, 1973, Fig. 3). Another distinguishing feature is the antagonism of LSD-inhibition in vasa by phentolamine. There is no evidence in the literature for such an interaction between phentolamine and guanethidine and in unpublished observations from this laboratory we have found that, in guinea-pig vasa, treatment with phentolamine does not prevent the paralysis produced by guanethidine.

In sympathetically innervated preparations LSD exerted two other actions, both of them post-synaptic, which sometimes masked its presynaptic effect. First, acting as an agonist, it gave rise to contractions due to a stimulation of post-synaptic α-adrenoceptors; in splenic strips this action was weak or absent, as had been found in guinea-pig vasa. Although, at first sight, this motor action of LSD may appear to resemble the contractile effect of guanethidine, the difference illustrated in Fig. 5 needs to be stressed: in the anococcygeus preparations taken from the reserpinized rats, in which the responses to field stimulation and to tyramine were paralysed, the contractile effect of guanethidine was lost but that of LSD persisted. This suggests that LSD is able to excite, directly, post-synaptic α-adrenoceptors in the smooth muscle. Innes (1962) also found that ergotamine and ergometrine, which bear considerable structural similarities to LSD, exerted a direct excitatory action on α-adrenoceptors in the smooth muscle of the dog retractor penis and in other adrenergically innervated organs. On the other hand, an unexplained discrepancy appears to exist between the foregoing findings and the preliminary report by Gillespie & McGrath (1974) that LSD-contractions were absent in rat anococcygeus preparations pre-treated with 6-hydroxydopamine.

The second masking effect of LSD was due to its action as an antagonist. Thus, it produced a blockade of post-synaptic α-adrenoceptors, which was most marked in the splenic strips but was feeble in the retractor penis and virtually absent in the anococcygeus, as in vasa. The weaker this post-synaptic α-blocking action of LSD, the easier it was to demonstrate its presynaptic, agonist effect. It is interesting to note that ergotamine has also been shown to possess both agonist and antagonist actions on α-adrenoceptors (Müller-Schweinitzer & Stürmer, 1974).

Inhibitory presynaptic adrenoceptors are also present in the cholinergic motor fibres to the longitudinal muscle of the guinea-pig ileum and have been classified by Paton & Vizi (1969) as α-receptors because of their susceptibility to phentolamine blockade. However, in our experiments on this preparation the type of presynaptic inhibition that is produced by LSD in sympathetically innervated muscles was absent. In higher concentrations LSD potentiated the twitches and acetylcholine-contractions, and antagonized the presynaptic inhibition produced by noradrenaline.

Thus, only the antagonist action of LSD could be demonstrated on the presynaptic α-adrenoceptors of these cholinergic fibres, which therefore appear to differ somewhat from the adrenoceptors present in sympathetic fibres.

An even greater difference was found previously in the inhibitory adrenoceptors present in the rabbit rectococcygeus preparation, as they could not be blocked by phentolamine, given alone or together with propranolol (Ambache, Killick & Zar, 1974). The present results show that LSD fails to produce presynaptic inhibition of the cholinergic motor transmission in this preparation, as in the longitudinal muscle of the guinea-pig ileum, and potentiates the responses to post-ganglionic stimulation and to acetylcholine. The mechanism underlying this potentiation needs further investigation.

After the preliminary abstract of this paper was published, some results obtained by Dr Angela Waterfield on plexus-containing longitudinal muscle preparations from guinea-pig ilea were cited in a communication by Hughes (1973). There is an unexplained discrepancy between her findings and ours: LSD, in comparable doses, depressed the twitches elicited by field stimulation in her preparations but never in ours.

As mentioned above, tyramine, which releases endogenous noradrenaline and so mimics the effects of sympathetic nerve stimulation, contracted all the adrenergically innervated preparations examined in this investigation. These results stand in marked contrast with the previous findings (Ambache *et al.* 1972) that in vasa from guinea-pigs of the strain used in this laboratory (Dunkin–Hartley albinos) tyramine, 11·6–58 μM, failed to mimic the motor transmission and always inhibited it. This and the fact that the motor transmission was inhibited also by cocaine led to the suggestion that the functional role of noradrenaline-releasing fibres in vasa may be inhibitory, i.e. to modulate the motor transmission; the present results show that such adrenergic fibres would also be susceptible to LSD and their noradrenaline-release would be reduced in its presence. In this context, it should be mentioned that Hughes (1973) found a reduction by LSD in the efflux, from stimulated guinea-pig vasa, of radioactivity derived from [^{3}H]tyrosine or [^{3}H]noradrenaline. The components of this efflux, which is partly derived from the circular muscle, were not rigorously identified by R_f values; nevertheless, Hughes (1973), finding some correlation between this reduction in efflux and the decreased mechanical response of the longitudinal muscle, has put this forward as evidence that the motor transmitter to the longitudinal muscle was, after all, noradrenaline, although Stjärne (1973) had drawn attention to the 'striking lack of correlation' between noradrenaline secretion and the longitudinal tension response. Moreover, although cocaine is known to increase noradrenaline

efflux (by blocking Uptake 1), and although it produces a 300-fold sensitization of motor responses to exogenous noradrenaline (Wakade & Krusz, 1972), it fails to augment the mechanical response of vasa to nerve stimulation (Ambache *et al.* 1972; Wakade & Krusz, 1972). These observations, too, are not consistent with Hughes' (1973) conclusion; and his findings with LSD, as ours, do not constitute evidence for or against adrenergic motor transmission in the vas deferens. Other equally valid explanations of his results can be advanced. They would, for instance, be compatible with the concept that the inhibitory action of LSD on presynaptic α-adrenoceptors produces a diminution in the release of neurotransmitter at both types of sympathetic endings, whether adrenergic or not.

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