THE EFFECTS OF LYSERGIC ACID DIETHYLAMIDE ON THE RESPONSE TO FIELD STIMULATION OF THE RAT VAS DEFERENS AND THE RAT AND CAT ANOCOCCYGEUS MUSCLES

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1   The effect of lysergic acid diethylamide (LSD) on the response to field stimulation *in vitro* of the rat vas deferens and anococcygeus muscle was examined.

2   LSD in concentrations from 10^{-9} to 10^{-6} M caused an increase in tone or rhythmic activity in both tissues, effects identical to those produced by guanethidine or tyramine. The motor effects of all three drugs were abolished by phentolamine 2×10^{-6} M. Methysergide 2×10^{-7} M given before LSD reduced the motor effect but was ineffective once the LSD contraction had developed.

3   LSD 10^{-9} to 10^{-6} M reduced and eventually abolished the response to motor adrenergic nerve stimulation in the anococcygeus muscle with no effect on the response to noradrenaline (NA) and no evidence of differential sensitivity according to the number of stimulating pulses. In the vas deferens LSD abolished the initial twitch component with no effect on the secondary slow contraction. LSD had no effect on the response to inhibitory nerve stimulation in the anococcygeus.

4   These results suggest that in the anococcygeus LSD closely resembles guanethidine in its effects as an adrenergic neurone blocking drug with indirect sympathomimetic actions. In the vas deferens these properties would explain the block of the initial twitch component in the motor response to field stimulation and the increase in rhythmic activity but do not explain the resistance of the secondary slow component of the motor response.

Introduction

Lysergic acid diethylamide (LSD) has been shown to produce at least two effects on smooth muscle preparations. First, it has been reported to cause contraction in the cat nictitating membrane (Thomson, 1958), the sheep umbilical artery (Dyer & Gant, 1973) and the rat and cat anococcygeus muscles (Gillespie & McGrath, 1974a). Secondly, LSD can inhibit the motor response to field stimulation of nerves in the rat and guinea-pig vas deferens (Ambache, Dunk, Verney & Aboo Zar, 1973) and recently this observation has been extended to the rat anococcygeus muscle (Ambache, Killick, Srinivason & Aboo Zar, 1973).

The pharmacology of motor transmission in the vas deferens presents several unusual features which have prompted Ambache & Aboo Zar, 1971 to suggest that the transmitter is not noradrenaline (NA). In contrast all of the evidence obtained in our laboratory suggests that the motor nerves in the anococcygeus are conventionally adrenergic.

We have, therefore, re-examined the effect of LSD on the rat vas deferens and compared this with the effect on the rat and cat anococcygeus muscles to try to determine whether the action of LSD in both organs is consistent with a motor adrenergic innervation. The findings indicate that LSD is both an indirect sympathomimetic and an adrenergic neurone blocking drug and that these two effects explain all of its actions on the anococcygeus. In the vas deferens, LSD also possesses these properties and this explains some but not all of its actions on this tissue. A preliminary report of some of these findings has been published (Gillespie & McGrath, 1974b).

Methods

Two types of preparation were used. Most work was on the isolated vas deferens or anococcygeus but in addition some *in vivo* experiments were
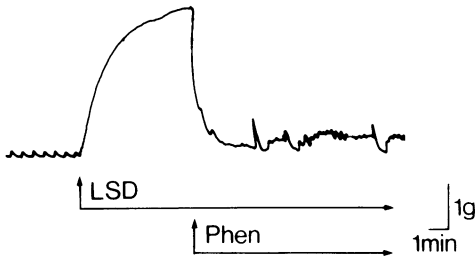
Figure 1 The motor response of the rat anococcygeus to lysergic acid diethylamide (LSD, 10^{-6} M) and its abolition by phentolamine (Phen, 10^{-6} M).

done on the pithed rat with a moveable spinal electrode to allow selective stimulation of spinal nerve outflows.

The isolation of the rat and cat anococcygeus muscles has already been described (Gillespie, 1972; Gillespie & McGrath, 1974a). Since the rat muscles are heavier and stronger in the male, only male animals were used but the cats were of either sex. The muscles were mounted in ring electrodes in a 10 ml bath containing Krebs-Henseleit solution at $37°$C and gassed with a mixture of 95% O_2 and 5% CO_2. Tension was measured with a Grass FTO3 isometric transducer and displayed on a Grass Polygraph. The rat vas deferens preparations were set up in a similar manner with the stimulating electrodes around the prostatic end of the muscle where the motor nerves enter the tissue. Field stimulation of the intramural nerves in these isolated tissues was with 1 ms pulses at supramaximal voltage at the frequencies indicated in the text.

In some preparations the terminal adrenergic nerve plexus was destroyed by exposure to relatively high concentrations of 6-hydroxy-dopamine (6-OHDA) added to the bath as described by Wadsworth (1973). The bath concentration of 6-OHDA used was 200 μg/ml; the drug was freshly prepared immediately before addition to the bath and it was left in contact with the tissue for 2 hours. The 6-OHDA was not protected by ascorbic acid since this itself was found to cause contraction of the anococcygeus muscle. The efficacy of this technique was shown by its ability to abolish the effect of motor nerve stimulation and of the indirect sympathomimetic, tyramine, with no reduction in the response to NA and also by the disappearance of fluorescent nerve fibres when the tissue was examined with the formaldehyde fluorescence technique of Falck & Hillarp.

The method of preparation of the pithed rat has previously been described (Gillespie, MacLaren

& Pollock, 1970). The moveable spinal electrode was placed to stimulate the nerve outflows between L1-L3 and again 1 ms pulses at supramaximal voltages were used. The method of recording tension from the anococcygeus muscles or vas deferens has also been described (Gillespie & McGrath, 1973). Systemic blood pressure was recorded from one femoral artery and drugs administered via a cannulated femoral vein.

The following drugs were used and concentrations are expressed as molar in the experiments *in vitro* and in grams of the base in the experiments in the pithed rat: guanethidine sulphate (Ciba), 6-hydroxydopamine hydrochloride (Calbiochem), lysergic acid diethylamide (Sandoz), methysergide bimaleate (Sandoz), noradrenaline bitartrate (Koch-Light), phentolamine mesylate (Ciba), tyramine hydrochloride (Sigma).

Results

Agonist action of LSD

In both the anococcygeus and the vas deferens, LSD in concentrations between 10^{-9} and 10^{-6} M caused contraction (Figures 1 and 2). In the anococcygeus the response was large, developed slowly in comparison with the effect of NA and was slow to reverse on washing the drug out of the bath. Repeated exposure to the drug resulted in a diminution in the response with no change in the response to NA. This tachyphylaxis also affected the indirect sympathomimetic action of tyramine and guanethidine. In the vas deferens LSD's ability to cause contraction was less dramatic and if the sensitivity of the recorder was appropriate to the maximum contractions induced by motor nerve stimulation then the motor effect of LSD could be overlooked. If however, the sensitivity was increased the LSD was shown in every preparation to cause a prolonged period of spontaneous mechanical activity (Figure 3). This pattern of activity was identical with that induced by two other indirect synpathomimetics, tyramine and guanethidine, as shown for guanethidine in Figure 2.

This motor effect in both the anococcygeus and the vas appeared to be due to an action on smooth muscle α-adrenoceptors since in both tissues it was abolished by phentolamine 2×10^{-6} M (Figures 1 and 2). Such an action could have been due either to a direct agonist interaction between LSD itself and the α-receptors or to an indirect sympatho-mimetic action of LSD and interaction of the released NA and the α-receptors. These alternatives were tested by examining the action of LSD on tissues previously exposed to 6-OHDA to destroy
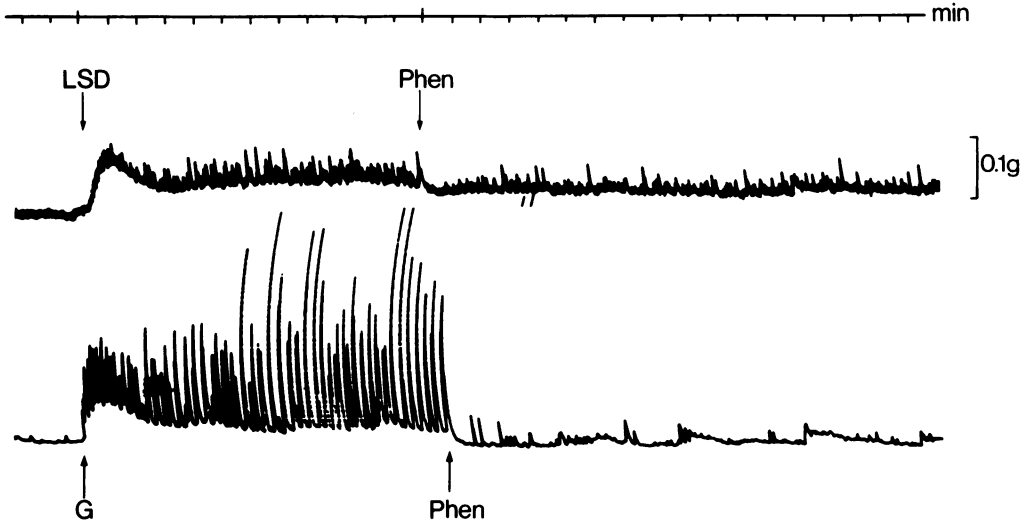
Figure 2 The motor effect of lysergic acid diethylamide (LSD, 10^{-7} M) and of guanethidine (G, 3×10^{-5} M) on the rat vas deferens and its abolition by phentolamine (Phen, 10^{-6} M). The rise in tone and increase in rhythmic activity is small and is clearly seen only if the recording sensitivity is high.
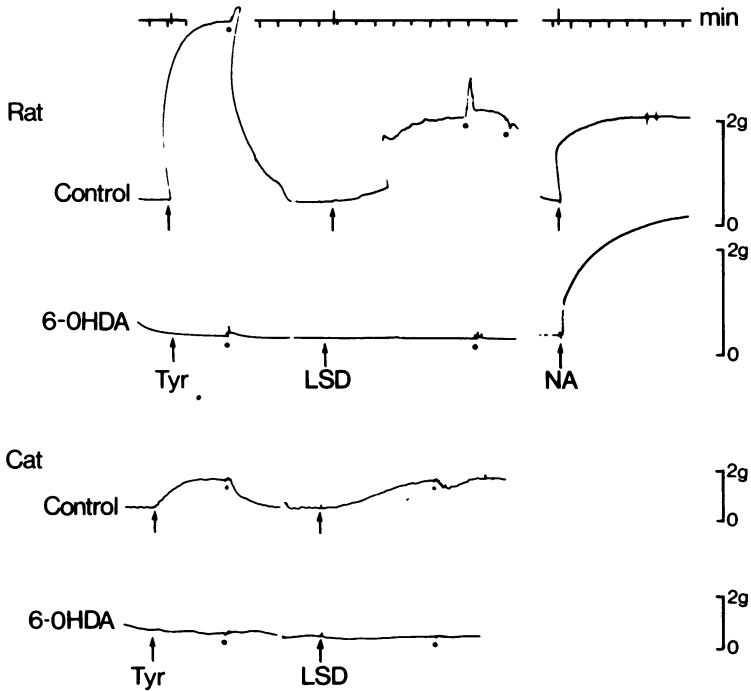

Figure 3 The effect of treatment with 6-hydroxydopamine (6-OHDA) on the response of the rat (upper two records) and the cat (lower two records) anococcygeus muscles to lysergic acid diethylamide (LSD, 2×10^{-8} M) and tyramine (Tyr, 10^{-5} M) and of the rat muscle to noradrenaline (NA, 10^{-7} M). Treatment with 6-OHDA abolished the motor response to both tyramine and LSD but potentiated the response to NA.
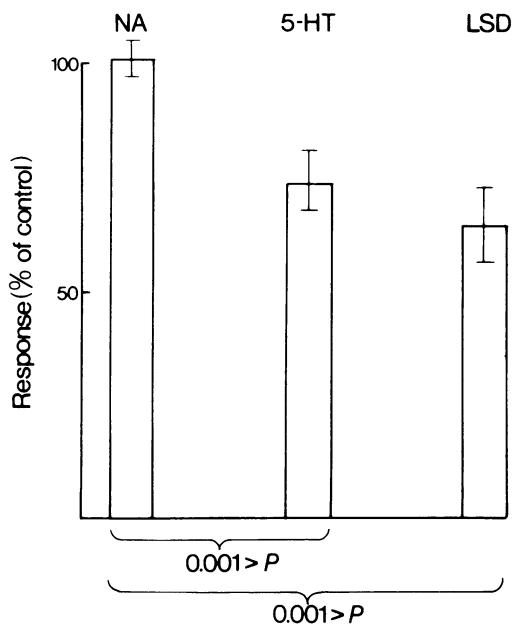
Figure 4 The effect of methysergide (2 x 10^{-7} M) on the motor response of the rat anococcygeus to noradrenaline (NA, 10^{-5} M), 5-hydroxytryptamine (5-HT, 10^{-5} M) and lysergic acid diethylamide (LSD, 10^{-7} M). The assessment of statistical significance shown below is by Student's *t* test.

the adrenergic nerves. In both the anococcygeus and the vas deferens motor effects in response to LSD, tyramine or guanethidine could not be obtained after treatment with 6-OHDA although the response to NA was still present and indeed enhanced. These results for the cat and rat anococcygeus are illustrated in Figure 3.

LSD is more commonly associated with the receptors for 5-hydroxytryptamine (5-HT) and for this reason we examined the effect of methysergide (2 x 10^{-7} M) on the motor effect of LSD. Unlike phentolamine, methysergide was found to be quite ineffective in abolishing the response to LSD once this was established. Methysergide, however, if added before LSD did prevent the development of contraction. The development of tachyphylaxis made this kind of experiment difficult to interpret using the control response to LSD in the same muscle. We, therefore, used paired muscles from the same animal mounted in separate baths, taking as control the response to LSD alone in one muscle and comparing this with the response to LSD in the presence of methysergide in the paired muscle. This method had the advantage that only one dose of LSD was used in each muscle but the disadvantage that the

variability in responses was increased. The effects of methysergide with this technique on the responses to LSD (10^{-7} M), 5-HT (10^{-5} M) and NA (10^{-5} M) on the rat anococcygeus are shown in Figure 4. The responses to NA were unaltered (101 ± 4% control) while those to LSD (65 ± 8% control) and to 5-HT (74 ± 7% of control) were inhibited. Methysergide itself in concentrations up to 10^{-5} M did not cause contraction in any of the tissues tested.

In the pithed rat intravenous injection of LSD 200 μg/kg caused contraction of both the anococcygeus and the vas deferens.

Effect of LSD on the responses to motor nerve stimulation

In the experiments described by Ambache *et al.*, 1973a & b, short trains of pulses were used to cause contraction and in the vas deferens in particular there is evidence of a differential sensitivity of the responses elicited by long and short trains of stimuli to pharmacological agents. For this reason we have examined the effect of LSD on the response to trains of different length varying from 1 to 100 stimuli but at a fixed frequency of 10 Hz. In the anococcygeus, LSD concentrations from 10^{-9} to 10^{-6} M inhibited the motor response to field stimulation in a manner identical to that of guanethidine though effective at lower concentrations than the latter drug (Figure 5). Unlike guanethidine all concentrations of LSD which reduced the response to nerve stimulation also caused contraction so that LSD commonly raised tone and simultaneously reversed the response to field stimulation to inhibition. This effect complicated the measurement of the reduction in the motor nerve response. This problem was overcome by increasing gradually the dose of LSD so that tachyphylaxis of its motor effect minimized the rise in base line. In this way the inhibitory effect of increasing doses of LSD on the motor nerve response was measured and the results are illustrated in Figures 6 and 7 for the rat anococcygeus and vas deferens. In the anococcygeus LSD almost completely inhibited the motor response and there was no evidence of a preferential effect on the response with short trains of stimuli.

The motor response of the vas deferens to field stimulation was more complex. The full response was seen with trains of stimuli greater than 20 pulses and consisted of an initial rapid contraction or 'twitch' followed by partial relaxation and then a secondary slower development of a maintained tension (Figure 6). Shorter trains of pulses favoured the twitch component of the response so that up to about 10 pulses there was no evidence
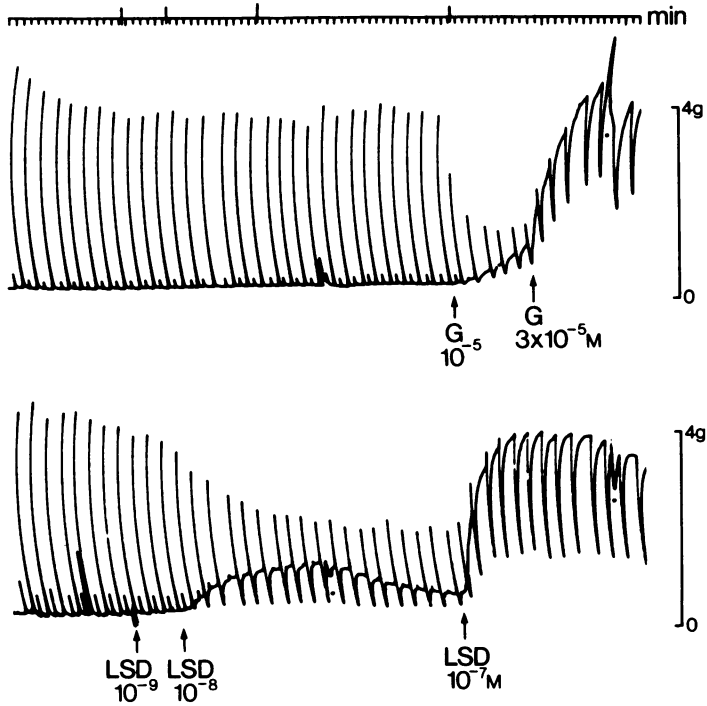
Figure 5   The effect of guanethidine (G) and lysergic acid diethylamide (LSD) on the response of the cat anococcygeus muscle to field stimulation. The nerves were alternatively stimulated with either a single stimulus or trains of 30 stimuli at 10 Hz. Both LSD and guanethidine inhibited the motor response, raised tone and reversed the response to field stimulation to inhibition.
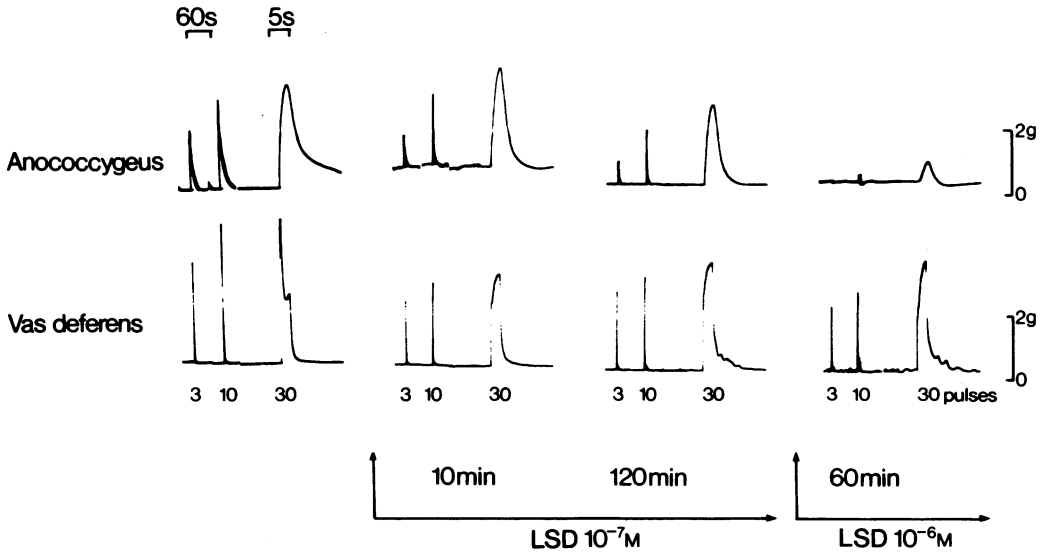

Figure 6   The effect of lysergic acid diethylamide (LSD) on the response of the rat anococcygeus muscle (upper records) and vas deferens (lower records) to stimulation with 3, 10 or 30 pulses at 10 Hz. In the anococcygeus the motor response is progressively inhibited until it is almost abolished. In the vas the control motor responses at 3 and 10 pulses consist of a single twitch but at 30 pulses the second slow component appears. LSD selectively inhibits the initial twitch component but potentiates the secondary response.
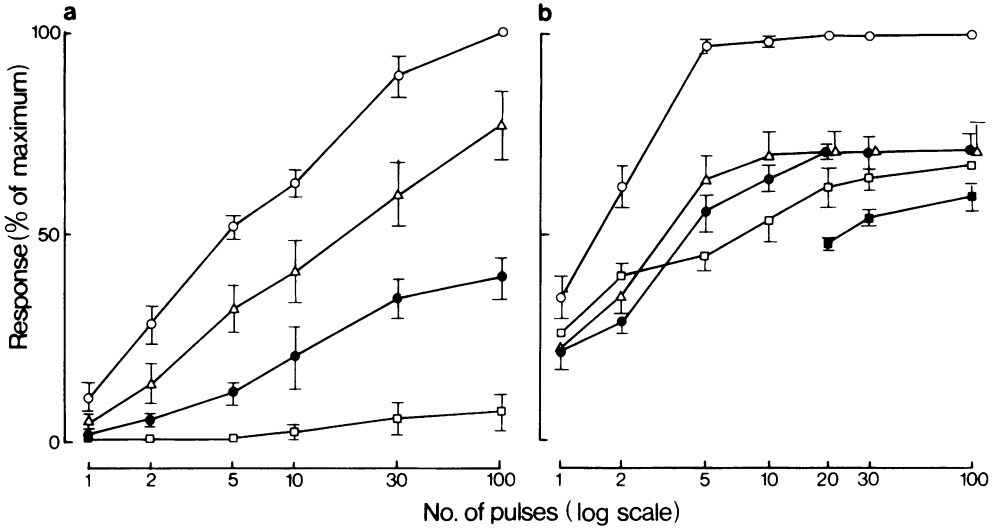
Figure 7   The effect of lysergic acid diethylamide (LSD) on the motor response of (a) the anococcygeus muscle and (b) the vas deferens to field stimulation at 10 Hz. The number of pulses is shown on the abscissae and the response as a percentage of the maximum on the ordinates. In the vas the amplitude of the initial spike response (○) and the secondary slow response (■) were measured in the control, in the anococcygeus there is only one component to the control response (○). The remaining graphs show the response in the presence of LSD 10^{-8} M (△), 10^{-7} M (●) and 10^{-6} M (□).

of a secondary component; with longer trains the amplitude of this secondary component increased though it never exceeded about 80% of the twitch response. LSD in concentrations from 10^{-9} to 10^{-6} M preferentially inhibited the initial twitch response with no effect on the secondary component or sometimes potentiating this (Figure 6). This effect of LSD was easy to demonstrate qualitatively but difficult to measure quantitatively since reduction of the 'twitch' caused it to merge with the secondary component and prevent further assessment. In Figure 7 we have separately measured, in control, the height of the twitch and of the secondary slow response; the secondary response was visible only with stimulus trains of 20 pulses or more. In the presence of LSD we measured the maximum response since we were unable to distinguish the two components. Two points of interest arise; first the response in the presence of LSD was always less than the control twitch amplitude and at low stimulus numbers where the twitch response predominates this appeared as a straight inhibition. With longer trains the response in the presence of LSD was less than the control twitch amplitude but greater than the secondary response which was potentiated (Figure 7). The second point was that, unlike the

anococcygeus, the inhibition in the vas deferens was less clearly related to the LSD concentration and had practically reached its maximum at 10^{-8} M.

In the pithed rat the maximum dose of LSD used, 200 μg/kg, had no effect on the motor response to nerve stimulation of the anococcygeus but did inhibit the first 'twitch' phase of the response in the vas deferens. Guanethidine 10 mg/kg completely abolished the entire motor response in both the anococcygeus and the vas deferens.

Effect of LSD on the response to inhibitory nerve stimulation

As Figure 5 shows, LSD was as effective as guanethidine in raising tone and reversing the response to field stimulation from contraction to inhibition.

If tone was first raised with guanethidine so as to convert the motor response to inhibition then the addition of LSD had no effect on these inhibitory responses as Figure 8 illustrates.
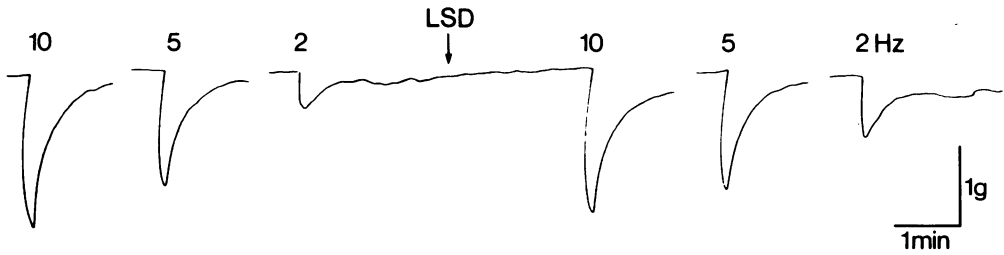
Figure 8   The absence of any effect of lysergic acid diethylamide (LSD, 3 x 10^{-7} M) on the inhibitory response to field stimulation of the rat anococcygeus muscle. In this experiment tone had been raised and the motor nerves blocked by the previous addition of guanethidine 3 × 10^{-5} M. The inhibitory response at three frequencies of stimulation shown above the records are unaltered by the addition of LSD.

Discussion

These results, particularly in the anococcygeus, strongly suggest that LSD can be regarded as an adrenergic neurone blocking agent with an indirect sympathomimetic action. The simultaneous comparison in Figure 5 of the action of guanethidine and LSD on a pair of muscles from the same animal shows identical effects with a rather similar time course. Even the smallness of the contractions produced in the vas deferens accords with an indirect sympathomimetic action since tyramine and guanethidine produce identically small responses in this tissue. That the contractions produced by LSD are due to the release of NA from adrenergic nerves is shown by their sensitivity to phentolamine and to 6-OHDA. That the reduction in the response to motor nerve stimulation is due to adrenergic neurone blockade is suggested by the undiminished response to NA and the lack of effect on the response to inhibitory nerve stimulation; it is also in agreement with the observation of Hughes (1973) that LSD reduces the nerve-stimulated release of NA from the adrenergic nerves of the guinea-pig vas deferens. 6-OHDA was employed to differentiate between the direct and indirect sympathomimetic action of LSD in preference to reserpine pre-treatment since a previous study (Gillespie & McGrath, 1974c) demonstrated that motor nerve responses could still be elicited from both anococcygeus and vas deferens even after the NA content had been reduced to very low levels. We suggest that the NA depletion of the adrenergic nerves by reserpine is incomplete and sufficient NA remains in these reserpine-treated animals both to maintain effective neurotransmission and an indirect sympathomimetic action. Our interpretation relies heavily on the ability of 6-OHDA *in vitro* to produce an effective sympathetic denervation. The technique has been used successfully in several other laboratories (Sachs, 1971; Furness,

1971; Wadsworth, 1973) and in the present investigation we have shown that it abolishes the response to motor nerve stimulation and to indirect sympathomimetic drugs while potentiating the response to NA. We have also found that the fluorescent adrenergic nerve terminals disappear and that they cannot be made to reappear by incubation with NA plus a monoamine oxidase inhibitor, as is possible after reserpine. The ability of LSD to exercise an indirect sympathomimetic action does not, of course, exclude the possibility that it can also in some tissues directly stimulate smooth muscle cells. An example of this is found in the experiments of Dyer & Gant (1973) who have shown that LSD can produce contraction of non-innervated umbilical blood vessels, an effect blocked by bromo-lysergic acid and therefore presumably mediated by 5-HT receptors on the smooth muscle. However, in the vas deferens and the anococcygeus, the complete absence of a motor effect after 6-OHDA and the complete block of the motor effect in normal tissue by phentolamine suggest that direct stimultion of the smooth muscle is negligible.

The ability of methysergide to reduce the motor effect of LSD and 5-HT without influencing the response to NA may indicate that the mechanism of LSD binding to the nerve membrane prior to uptake involves a 5-HT receptor. This might also explain why methysergide is ineffective if added after the response to LSD has fully developed. If the LSD is now inside the nerve varicosity then interference with uptake will not prevent its sympathomimetic action. On this interpretation phentolamine blocks the action of LSD post-synaptically at the α-adrenoceptors while methysergide acts pre-synaptically to block receptors involved in the uptake of LSD.

The hypothesis we put forward will explain all of the actions of LSD on the rat and cat

anococcygeus and also the ability to cause contraction of the vas deferens. It does not explain the obvious difference in the vas deferens between guanethidine which abolishes the entire motor nerve response and LSD which inhibits only the initial twitch component. The origin of the two components of this response in the vas is obscure; drugs such as reserpine and phentolamine which have a predictable action on other adrenergically innervated tissue produce a selective block of one or other component of the response in the vas deferens (Swedin, 1971; Gillespie & McGrath,

1974c) and LSD appears to be in a similar category. These exceptions we feel are more a reflection of the unusual nature of the motor response in the vas deferens than a criticism of the mode of action of such drugs on adrenergic neuro-effector transmission generally.

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