Short communications

Inhibition of noradrenaline release by lysergic acid diethylamide

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Lysergic acid diethylamide (LSD) inhibits the release of labelled noradrenaline from the guinea-pig vas deferens during intramural nerve stimulation and causes a corresponding reduction in the contractions of the smooth muscle. These effects of LSD are most prominent at low stimulus frequencies and they are prevented by treatment with phentolamine. It is concluded that LSD inhibits noradrenaline release by interacting with presynaptic α-adrenoceptors.

Attention has focussed mainly on two aspects of the pharmacology of lysergic acid diethylamide (LSD), firstly its ability to antagonize the effects of 5-hydroxytryptamine at peripheral (Gaddum, 1957) and central sites (Boakes, Bradley, Briggs & Dray, 1970), and secondly the possibility that it may actually stimulate central tryptaminergic receptors by mimicking the action of endogenous 5-hydroxytryptamine (Andén, Corrodi, Fuxe & Hökfelt, 1968). I wish to report a further pharmacological action of LSD, namely the ability to inhibit noradrenaline release from adrenergic motor nerves in the guinea-pig vas deferens. This work was prompted by the observation that contractions of the vas deferens elicited by intramural nerve stimulation were reduced by LSD (Ambache, Dunk, Miall & Aboo Zar, 1971), although the authors did not consider this effect to be mediated via an adrenergic mechanism.

Methods.—Isolated preparations of the desheathed vas deferens were incubated in Krebs solution at 36°C with either (−)-[^{3}H]-noradrenaline (100 ng/ml, 40 μCi/μg; Amersham Radiochemicals) or L-3,5-[^{3}H]-tyrosine (0·25 μg/ml, 221 μCi/μg; Amersham Radiochemicals) for 1 or 2 h respectively. At the end of the incubation period the vas was rinsed in fresh Krebs solution (changed every 10 min) for 2 hours. The tissue was finally suspended in a 3 ml organ bath maintained at 36°C and gassed with 95% O_2:5%CO_2. The intramural nerves were excited by a field current (1 ms rectilinear pulses of supramaximal strength) applied between platinum electrodes set in the top and bottom of the bath. Contractions were monitored with an isometric transducer coupled to a Grass Polygraph. To study transmitter release the vas was stimulated at 0·2 Hz for 3·5 min every 15 minutes. The bath fluid was removed 30 s after the end of each stimulation period and the labelled catecholamines were isolated by alumina column chromatography (Boadle-Biber, Hughes & Roth, 1970). The activity of the alumina eluate was determined in a Packard liquid scintillation counter (Model 2425). Basal samples for the 4 min preceding the onset of each stimulation period were taken and treated as described above, the basal radioactivity was deducted from the stimulated efflux.

Results.—LSD tartrate caused a rapid and prolonged inhibition of the contractions to electrical stimulation. The effect was most marked at low stimulus frequencies (0·1 to 0·5 Hz), and at frequencies above 5 Hz was apparent only for the first 5 to 15 pulses. A 50% inhibition of the twitch height at 0·1 Hz was obtained with a concentration of 18 ± 4 ng/ml ($n=4$), and complete inhibition with 50–100 ng/ml (2 min contact time). The $T_{\frac{1}{2}}$ for recovery of the twitch at 0·1 Hz after complete inhibition was 22 ± 5 minutes. Contractions of the vas deferens elicited by noradrenaline were either unaffected or slightly potentiated by LSD. Noradrenaline itself (0·1–1·0 μg/ml) first potentiated and then depressed the response to electrical stimulation at 0·1 Hz.

Electrical stimulation of the vas for 3·5 min at 0·2 Hz caused a reproducible increase in the efflux of [^{3}H]-catecholamines above basal levels (Figure 1). The basal efflux of label was unaffected by LSD (50 ng/ml) but the stimulation efflux was reduced to 20–40% of the pre-drug value (Figure 1). The contractions and the stimulated efflux gradually recovered after washing out the LSD and reintroduction of the drug caused a further inhibition of the contraction and efflux. The same result was obtained irrespective of whether [^{3}H]-noradrenaline or [^{3}H]-tyrosine was used to

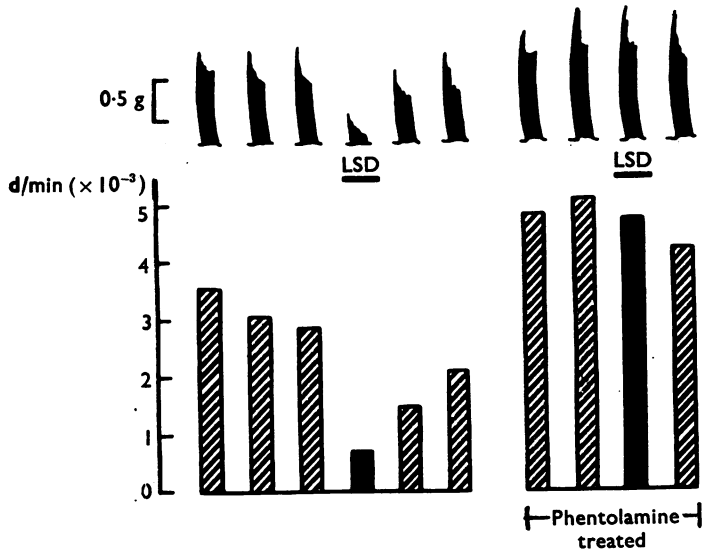

FIG. 1. Antagonism of lysergic acid diethylamide (LSD) by phentolamine. Vas deferens labelled with [^{3}H]-noradrenaline and field-stimulated as described in **Methods**. The upper part of the diagram shows the tension developed in the vas during periods of stimulation. The lower part of the diagram shows the increased levels of ^{3}H-labelled material released into the bath during periods of stimulation in the absence (hatched columns) and in the presence (filled columns) of LSD (50 ng/ml). For the experiments recorded on the right-hand side, phentolamine (2 μg/ml) was present in the bathing solution.

label the tissue stores. The inhibitory effect of LSD was abolished by phentolamine (2 μg/ml) which itself markedly increased the contractions and efflux of label during electrical stimulation (Figure 1).

Five experiments were carried out by Dr. Angela Waterfield on the isolated longitudinal muscle-myenteric plexus preparation of the guinea-pig ileum. Contractions elicited by field stimulation at 0·1 Hz were rapidly depressed by LSD, a 50% depression being obtained with 100 ng/ml. Contractions of the myenteric plexus elicited by acetylcholine were not significantly affected by LSD.

Discussion.—These results confirm and extend those of Ambache *et al.* (1971) and Ambache, Dunk, Verney & Aboo Zar (1973); however, these workers consider that the motor innervation of the vas is non-adrenergic. The present results support the classical concept that noradrenaline is the motor transmitter in the vas deferens. The parallel reduction of both the contractions and label efflux during stimulation are most likely causally related. Although [^{3}H]-noradrenaline might be taken up and released by non-adrenergic nerves, this is most unlikely in the case of [^{3}H]-noradrenaline derived from labelled tyrosine.

The results with phentolamine may provide an explanation for the potent and prolonged effect of LSD. It has been suggested that noradrenaline release may be subject to a type of feed-back control exerted by noradrenaline itself on presynaptic α-adrenoceptors (Farnebo & Malmfors, 1971; Starke, 1972). LSD may mimic this effect by combining with the presynaptic receptor sites. This would explain why the effect of LSD is limited to very low frequencies or short trains of pulses, for under these conditions the intrinsic feed-back control would be less active than when greater amounts of transmitter are released at higher frequencies (Hughes, 1972) or longer trains of pulses (Hughes & Roth, 1972). Noradrenaline itself can depress the contractions at 0·1 Hz and this supports the concept of feed-back inhibition. The preliminary results in the myenteric plexus provide additional support for this hypothesis, for there is little doubt that acetylcholine is the transmitter here and it has been shown that acetylcholine release is inhibited by

α-adrenoceptor stimulation at this site (Kosterlitz, Lydon & Watt, 1970).

It is possible that phentolamine may antagonize the effects of LSD not by preventing access to a presynaptic receptor site but by some other action, possibly consequent upon the increased efflux of transmitter. However, if LSD and phentolamine do interact at the same receptor then we may have to re-evaluate our concept of α-adrenoceptors. LSD has only a weak action on vascular α-adrenoceptors (Hoffman, 1968) and did not cause contractions of the guinea-pig vas deferens; the presynaptic adrenoceptor would then appear to differ from the classical α-adrenoceptor in terms of its agonist specificity.

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