LSD, MESCALINE AND SEROTONIN INJECTED INTO MEDIAL RAPHE NUCLEUS POTENTIATE APOMORPHINE HYPERMOTILITY *

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Microinjections of LSD (0.05 μg), mescaline (0.5 μg) and serotonin (10 μg) into the medial raphe nucleus of rats resulted in a strong potentiation of apomorphine (1 mg/kg i.p.)-induced hypermotility. The potentiating effect of LSD or serotonin was suppressed by simultaneous injections of methysergide (0.05 μg) or cyproheptadine (0.05 μg) into the medial raphe nucleus. The same doses of LSD injected into the dorsal raphe nucleus and of LSD and mescaline injected into the nucleus accumbens failed to influence locomotor activity, whereas injections of higher doses of LSD and mescaline into the nucleus accumbens inhibited spontaneous and apomorphine-stimulated locomotor activity. It is concluded that the potentiating effect of systemically administered low doses of hallucinogens was triggered by preferential actions on the serotonergic system in the medial raphe nucleus.

Medial and dorsal raphe nucleus Locomotor activity Serotonin Mescaline LSD Serotonin antagonists

1. Introduction

A common property of hallucinogens like lysergic acid diethylamide (LSD), dimethyltryptamine (DMT), and mescaline seems to be their rather specific action on the central serotonin (5-HT) system (for review see Boarder, 1977). Hallucinogens inhibit the firing rate of 5-HT containing midbrain raphe neurons after their intravenous injection in low doses and like 5-HT, after microiontophoretic administration also, probably by an action on somatodendritic 5-HT receptors (Aghajanian et al., 1970; Haigler and Aghajanian, 1977). In agreement with this suppression of the spontaneous firing rate and the impulse flow in 5-HT pathways, to show which new cytofluorimetric technique has recently been used, others have similarly demonstrated an increased level of 5-HT within the cell bodies of the dorsal and medial raphe nuclei after intraperitoneal injections of LSD, DMT and mescaline (Geyer, 1980) and a decrease in the turnover rate of 5-HT by LSD (Lin et al., 1969; Schubert et al., 1970, Kehr, 1977).

Previously, we have shown that low doses of LSD, mescaline, and DMT administered i.p. in rats potentiated locomotor hyperactivity induced by dopaminergic drugs (Fink et al., 1979). The potentiating effect of LSD was abolished by the 5-HT antagonist cyproheptadine and as has been shown in further studies also by danitracene, mianserin and pizotifen (Gold et al., 1980). Corresponding to this potentiating effect of hallucinogens, a decrease in functional brain 5-HT after electrolytic lesions of the medial raphe nucleus also caused an increase in amphetamine-induced locomotor hyperactivity, whereas lesions of the dorsal raphe nucleus remained ineffective in this respect (Jacobs et al., 1975; Geyer et al., 1976).

These electrophysiological, neurochemical and behavioural findings provide some evidence that the potentiating effect of hallucinogens may be triggered at the level of the medial raphe nucleus

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but definite conclusions cannot be drawn. We therefore investigated the effects of LSD, mescaline and 5-HT on spontaneous locomotor activity and apomorphine-induced hypermotility and the presumed antagonistic actions of methysergide and cyproheptadine following their microinjection into the medial raphe nucleus and that of LSD into the dorsal raphe nucleus of conscious rats.

The most suitable site for studying the interaction between the 5-HT and the mesolimbic dopamine system especially in regard to the potentiation of apomorphine-induced hypermotility seems to be the nucleus accumbens which is known to be mainly involved in the performance of locomotor effects and on the other hand exhibits a dense 5-HT innervation. This assumption is supported by the finding that dopaminergic-induced hyperactivity was antagonized by 5-HT and potentiated by methysergide following microinjections of these drugs into the nucleus accumbens (Costall and Naylor, 1978; Carter and Pycock, 1978; Costall et al., 1976). It can be assumed that depending on the site of action at somatodendritic or postsynaptic 5-HT receptors opposite behavioural effects may appear. Therefore we also examined the effect of LSD and mescaline after microinjections into the nucleus accumbens.

2. Materials and methods

2.1. Animals

The experiments were performed on male Wistar rats (VEB Versuchstierproduktion Schönwalde) weighing 140 ± 10 g. They were housed in groups of 10 per cage at a room temperature of $22 \pm 2°C$ and a 12 h light-dark schedule. The rats received food and water ad libitum.

2.2. Intracerebral injection technique

Intracerebral injections were made by means of platforms with fixed guide cannulae (Westermann and Andreas, 1971). For injections into the medial and the dorsal raphe nucleus platforms were used with one guide cannula fixed at an angle of 30° and 20°, respectively, to avoid damage to the saggital sinus and to leave the dorsal raphe nucleus intact while injecting into the medial raphe nucleus. The platform for bilateral injections into the nucleus accumbens had two vertically fixed guide cannulae.

Rats were anesthetized with sodium hexobarbitone, 140 mg/kg i.p. and the platforms were screwed on the skull. On the next day an injection cannula with an outer diameter of 0.23 mm was inserted slowly through the guide cannula into the target nucleus. The coordinates of the cannula tips were, according to König and Klippel (1963), for medial raphe nucleus $A = 0.35$; $L = 0$; $V = 2.6$; for dorsal raphe nucleus $A = 0.16$; $L = 0$; $V = 0.8$; and for nucleus accumbens $A = 9.4$; $L = 1.3$; $V = 1.0$. Drugs or 0.9% NaCl were administered to conscious hand-held animals by means of a Hamilton μl syringe (CR700-20) in a volume of 1 μl over 30 sec with a further 30 sec allowed for deposition of drug. Rats were used only once and killed after the experiment for histological examination.

2.3. Measurement of locomotor activity

The behavioural experiments were carried out between 8.00 a.m. and 11.00 a.m. in a soundproofed diffusely illuminated room maintained at a temperature of $22 \pm 2°C$. The rats were allowed to adapt to this room for 2 h. The intracerebral injection was followed by an i.p. injection. Immediately after drug administration the rat was returned to its home cage for 7 min and then transferred to the middle of an open-field cage. The white open-field cage consisted of a 1 m $\times$ 1 m area divided into 36 equal squares and surrounded by a 40 cm high wall.

The locomotor activity of the rat in a new environment was measured by the number of squares crossed during a 5 min observation period. Aberrant behaviour patterns were noted, such as stereotypies, straub tail etc.

2.4. Drugs

For intracerebral injections cyproheptadine-HCl (Sharp and Dohme), LSD (lysergic acid diethylamide tartrate) (SPOFA), mescaline sulfate (Merck), methysergide hydrogen maleate (Sandoz),

and serotonin creatinine sulfate (Reanal) were dissolved in 0.9% NaCl. For i.p. injections apomorphine-HCl (SPOFA) was dissolved in saline and administered in a volume of 1 ml/100 g body weight. Controls were treated intracerebrally and/or systemically with saline. All drug solutions were freshly prepared. All doses were calculated as the salt. Values are presented as the mean ± standard error of the mean (S.E.M.). Significance was assessed using the nonparametric Mann-Whitney test.

3. Results

3.1. Effect of LSD injected into the medial and dorsal raphe nucleus on spontaneous and apomorphine-stimulated locomotor activity

The operation, the platform screwed onto the skull as well as injections of saline into the medial raphe nucleus did not influence spontaneous locomotor activity and locomotor hyperactivity induced by 1 mg/kg apomorphine i.p. Locally and systemically saline-treated animals crossed about 40 squares during the 5 min observation period. Following systemic administration of 1 mg/kg apomorphine and local saline injections, locomotor activity was increased, varying between 80 and 100 squares crossed. Apomorphine induced headwaving and continuous sniffing in both operated and unoperated animals while they were in motion.

LSD injected into the medial raphe nucleus in a dose of 0.05 μg failed to influence spontaneous locomotor activity, but caused a strong potentiation of apomorphine-induced hypermotility. The animals crossed about 160 squares and ran quickly during the entire observation period (fig. 1). Aberrant behaviour patterns, e.g. straub tail, piloerection, hindlimb abduction, and a high degree of

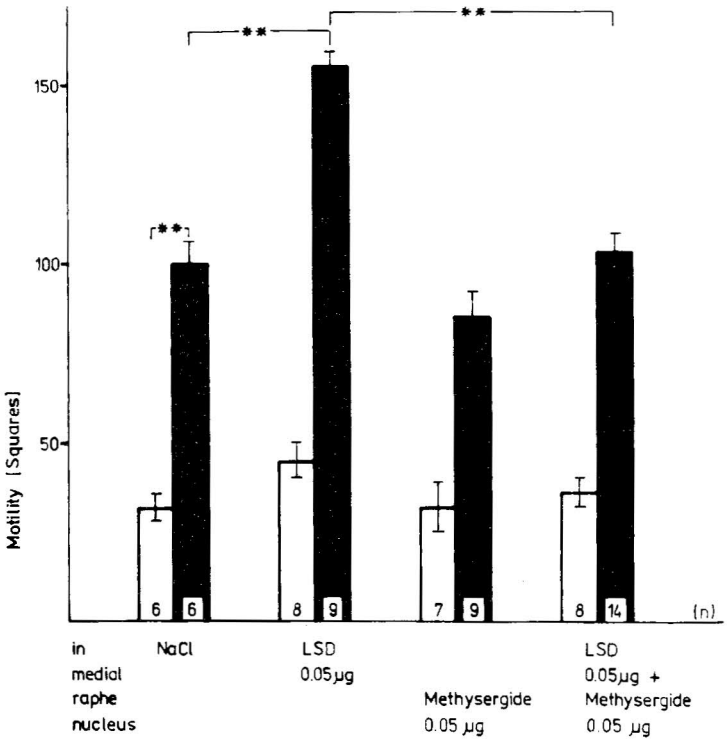


Fig. 1. Effect of LSD and methysergide injected into the medial raphe nucleus on spontaneous and apomorphine-stimulated locomotor activity. Crossed squares of an open field were counted for 5 min, 8 min after intra-raphe administration and 7 min after i.p. injection of the drugs. Columns represent means ± S.E.M. □ NaCl i.p.; ■ apomorphine, 1 mg/kg i.p. n = number of animals. ** $P < 0.005$, Mann-Whitney test.

alertness occurred. The doubling of the dose of LSD did not further increase locomotor hyperactivity (not shown). Injections of 0.05 μg LSD into the dorsal raphe nucleus remained ineffective, e.g. neither spontaneous nor apomorphine-stimulated locomotor activity were influenced and aberrant behaviour patterns did not occur (fig. 2).

3.2. Influence of methysergide and cyproheptadine on LSD-induced potentiation effect

Methysergide and cyproheptadine in a dose of 0.05 μg injected into the medial raphe nucleus produced a weak, but not significant decrease of spontaneous locomotor activity and apomorphine-induced hypermotility, respectively (figs. 1, 3). After the simultaneous administration of 0.05 μg methysergide or 0.05 μg cyproheptadine and 0.05 μg LSD in a volume of 1 μl into the medial raphe nucleus, the potentiating effect of LSD was completely abolished (figs. 1 and 3).

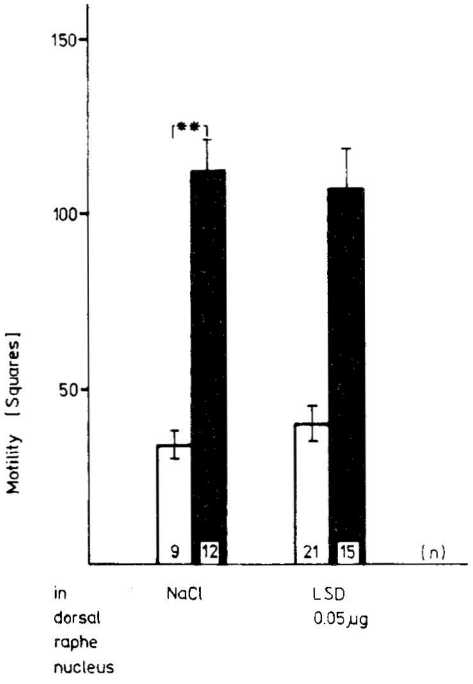


Fig. 2. Effect of LSD injected into the dorsal raphe nucleus on spontaneous and apomorphine-stimulated locomotor activity. For further explanation see legend of fig. 1. □ NaCl i.p.; ■ apomorphine, 1 mg/kg i.p. ** $P<0.005$, Mann-Whitney test.

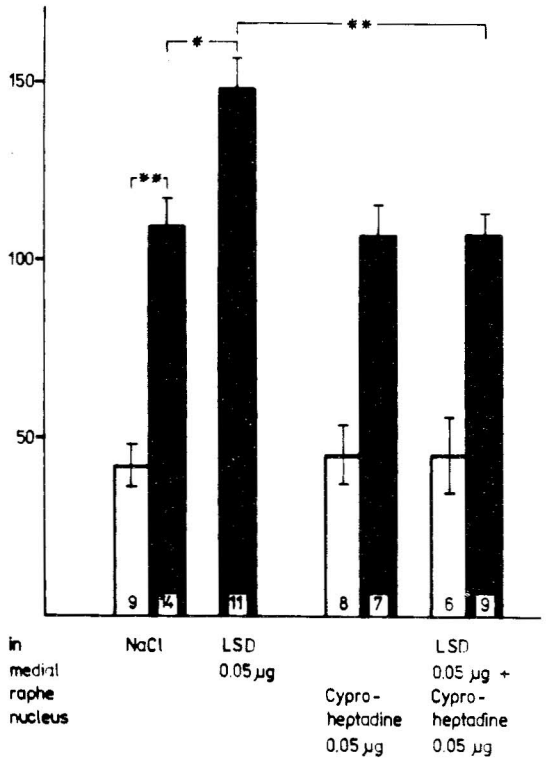


Fig. 3. Influence of cyproheptadine injected into the medial raphe nucleus on LSD-induced potentiating effect. For further explanation see legend of fig. 1. *Ordinate*: motility (squares); n is shown within the columns. □ NaCl i.p.; ■ apomorphine, 1 mg/kg i.p. ** $P<0.005$, * $P<0.01$, Mann-Whitney test.

3.3. Effect of mescaline and serotonin injected into the medial raphe nucleus on spontaneous and apomorphine-stimulated locomotor activity

Doses of 0.5 μg mescaline and 10 μg 5-HT, respectively, injected into the medial raphe nucleus failed to influence spontaneous locomotor activity but caused a pronounced potentiation of apomorphine-induced locomotor stimulation. Following both drug combinations the animals crossed 140 squares (figs. 4, 5). Aberrant behaviour patterns as described above for LSD were elicited only by mescaline. The potentiating effect of 5-HT was completely suppressed by a simultaneous microinjection of 0.05 μg methysergide.

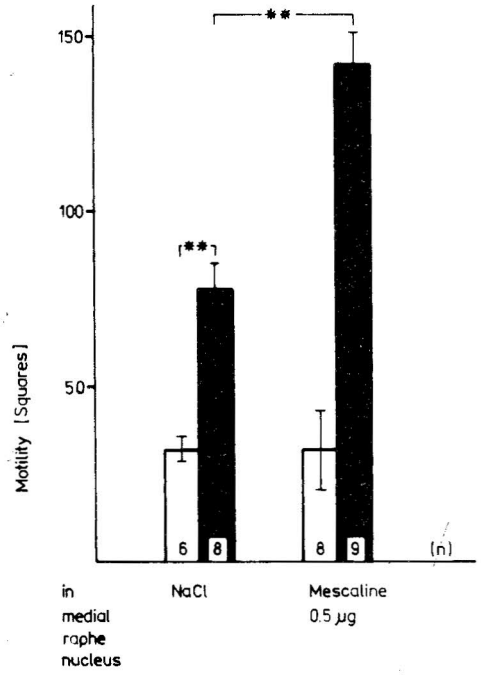
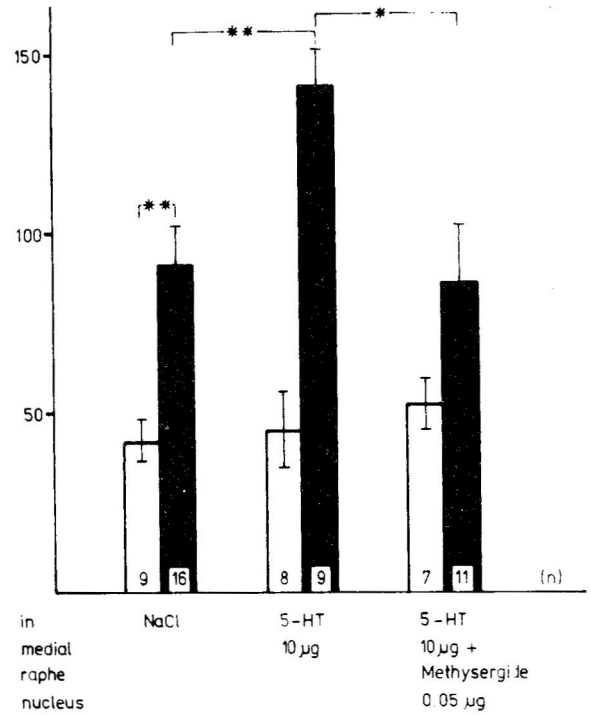


Fig. 4. Effect of mescaline injected into the medial raphe nucleus on spontaneous and apomorphine-stimulated locomotor activity. For further explanation see legend of fig. 1. *Ordinate*: motility (squares). □ NaCl i.p.; ■ apomorphine, 1 mg/kg i.p. ** $P<0.005$, Mann-Whitney test.



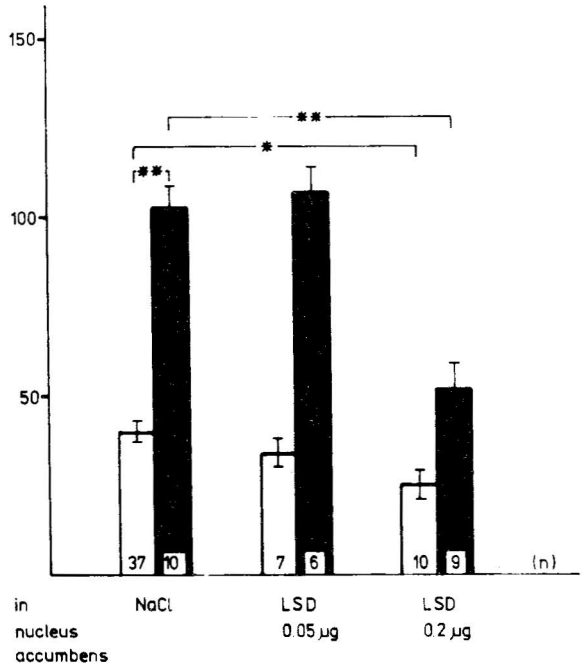


Fig. 6. Effect of LSD injected into the nucleus accumbens on spontaneous and apomorphine-stimulated locomotor activity. Crossed squares of an open field were counted for 5 min, 8 min after intra-accumbens administration and 7 min after i.p. injection of the drugs. Columns represent means ± S.E.M. *Ordinate*: motility (squares). □ NaCl i.p.; ■ apomorphine, 1 mg/kg i.p. n = number of animals. ** $P<0.005$, * $P<0.025$, Mann-Whitney test.

3.4. Effect of LSD and mescaline injected bilaterally into the nucleus accumbens on spontaneous and apomorphine-stimulated locomotor activity

Doses of LSD and mescaline which were strongly potentiating when injected into the medial raphe nucleus did not influence the locomotor activity of saline or apomorphine-treated animals. Injections of 0.2 µg LSD and 5 µg mescaline resulted in a decrease of spontaneous locomotor activity and a marked inhibition of apomorphine-induced hypermotility (figs. 6, 7).

Fig. 5. Effect of 5-HT and methysergide injected into the medial raphe nucleus on spontaneous and apomorphine-stimulated locomotor activity. For further explanation see legend of fig. 1. □ NaCl i.p.; ■ apomorphine, 1 mg/kg i.p. ** $P<0.005$, * $P<0.025$, Mann-Whitney test.

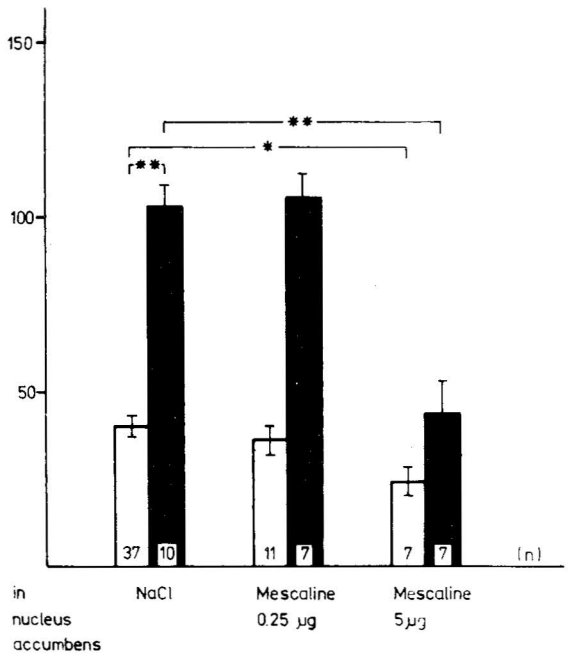


Fig. 7. Effect of mescaline injected into the nucleus accumbens on spontaneous and apomorphine-stimulated locomotor activity. For further explanation see legend of fig. 5. *Ordinate*: motility (squares). □ NaCl i.p.; ■ apomorphine, 1 mg/kg i.p. ** $P<0.005$, * $P<0.05$, Mann-Whitney test.

4. Discussion

We have previously demonstrated that low doses of LSD and mescaline given i.p. to rats failed to influence spontaneous locomotor activity but caused a strong potentiation of the hypermotility induced by apomorphine and amphetamine (Fink et al., 1979) or by dopamine injected into the nucleus accumbens (Oelssner et al., 1980). Quite similar potentiating effects were obtained in the present study with microinjections of LSD and mescaline into the medial raphe nucleus. The action seems to have been restricted to the nucleus because seven minutes after administration of [^{3}H]LSD into the medial raphe nucleus there was only sparse labelling of neighbouring brain structures, e.g. ventral tegmentum (K. Drescher, personal communication). Furthermore, LSD remained ineffective after injection into the dorsal raphe nucleus which was comparable with the ineffectiveness of lesions of this nucleus whereas lesions of the medial raphe nucleus caused an increased locomotor response to amphetamine (Jacobs et al., 1975; Geyer et al., 1976). Therefore the potentiation effect of LSD, and probably that of mescaline too, appears to be triggered by a rather specific action on the medial raphe nucleus.

The corresponding potentiating effects following microinjections of LSD and the physiological transmitter 5-HT, respectively, into the medial raphe nucleus and the abolition of these effects by low doses of 5-HT antagonists after systemic as well as intra-raphe administration provide evidence for a causal involvement of the 5-HT transmission system and also of 5-HT receptors. Electrophysiological studies (Haigler and Aghajanian, 1977) and binding studies with [^{3}H]LSD (Bennett and Aghajanian, 1975) and [^{3}H]5-HT (W. Oelssner jr., personal communication) point to the existence of 5-HT receptors in the midbrain raphe nuclei and an action of intra-raphe-injected LSD and 5-HT on these receptors is probable. The location and functional relevance of these 5-HT receptors, however, still remains unclear. This also applies to the marked antagonistic activity of methysergide and cyproheptadine in our experiments whereas no antagonism could be found towards LSD-induced suppression of the firing rate of raphe neurons (Haigler and Aghajanian, 1977). However, a causal relationship between electrophysiological and pharmacological phenomena seems to be doubtful as may be concluded from the finding that LSD effects on behaviour are dissociated from raphe unit activity (Trulson and Jacobs, 1979). Because of the presence of other than 5-HT-containing neurons and terminals in the midbrain raphe nuclei (Saavedra et al., 1976; Ochi and Shimizu, 1978; McGeer et al., 1979) indirect actions of hallucinogens on the 5-HT system cannot be excluded, especially in the case of mescaline. Further studies are now under way to get more insight into the presumed importance of the 5-HT system and its possible interaction with other transmission systems in the medial raphe nucleus.

Nevertheless electrophysiological, neurochemical and histochemical findings suggest an inhibitory effect of hallucinogens on 5-HT functions.

This effect is believed to depend on 5-HT-like actions on somatodendritic 5-HT receptors in the raphe nuclei (see Introduction). Furthermore an inhibitory influence of the 5-HT system on dopaminergic pathways has been demonstrated in numerous behavioural studies. Lowering of 5-HT availability by different manipulations, e.g. insufficient availability of the precursor l-tryptophan (Hollister et al., 1976), inhibition of 5-HT synthesis (Mabry and Campbell, 1973), electrolytic lesions (Neill et al., 1972; Jacobs et al., 1975) or chemical lesions (own unpublished results) of the medial raphe nucleus and microinjections of 5-HT antagonists into the nucleus accumbens (Carter and Pycock, 1978), causes a potentiation of dopaminergic-induced hypermotility whereas an increase in 5-HT activity, e.g. by microinjections of 5-HT into the nucleus accumbens (Costall and Naylor, 1978), induces opposite effects.

Our results are in accordance with these findings and provide an additional hint as to the postulated inhibitory modulation of dopaminergic functions by the 5-HT system. Obviously microinjections of LSD, mescaline and 5-HT into the medial raphe nucleus cause a decrease in 5-HT activity and consequently potentiation of apomorphine-induced hypermotility. On the other hand microinjections of LSD and mescaline into the nucleus accumbens increase the inhibitory 5-HT input probably by an action on postsynaptic 5-HT receptors which results in an inhibition of spontaneous and apomorphine-stimulated motility. Our findings do not support conclusions about an agonistic action of LSD on postsynaptic dopamine receptors which has been demonstrated only after induction of supersensitivity of postsynaptic dopamine receptors (Kelly and Iversen, 1975). Interestingly, doses of LSD and mescaline which were effective after raphe injections were ineffective after intra-accumbens administration and higher doses were needed to get clear-cut effects. This difference in potency according to whether the effects were exerted on the origin or on the terminal areas of the 5-HT system agrees well with electrophysiological data on a preferential action of systemically administered hallucinogens on somatodendritic 5-HT receptors (Haigler and Aghajanian, 1977); it also appears suitable to explain the pronounced potentiation of dopaminergic-induced hypermotility after i.p. injections of rather low doses of LSD (Fink et al., 1979) whereas postsynaptic-induced effects, e.g. the 'serotonin syndrome' predominate after higher doses.

Some components of this syndrome are believed to be triggered in the lower brain stem and in the spinal cord (Jacobs and Klemfuss, 1975). In our studies we observed the induction of these aberrant behavioural patterns (Straub tail, hindlimb abduction etc.) also by intra-raphe administered LSD and mescaline but only in apomorphine-pretreated animals. The failure of intra-raphe-administered 5-HT to provoke this syndrome points to an additional effect of hallucinogens, possibly due to a sensitization of other than 5-HT receptors as has been shown recently by McCall and Aghajanian (1980) in electrophysiological studies.

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