Br. J. Pharmac. (1969), **35**, 243–252.

A study of the role of noradrenaline in behavioural changes produced in the rat by psychotomimetic drugs

M. F. SUGRUE*

Division of Experimental Pharmacology, Institute of Physiology, The University, Glasgow, W.2

1. LSD-25, psilocybin and JB-329 reduced the noradrenaline content of the rat hypothalamus.

2. All three drugs affected the acquisition of a conditioned avoidance response, LSD-25 and psilocybin retarding and JB-329 enhancing the acquisition. With the exception of JB-329, doses affecting the acquisition of a conditioned avoidance response were lower than those required to decide hypothalamic noradrenaline concentrations. The time of peak drug effect on the acquisition of a conditioned avoidance response occurred approximately 1.5 hr after injection as opposed to 3 hr in the case of noradrenaline content.

3. The amount of LSD-25, psilocybin and JB-329 necessary to elicit gross behavioural excitation was similar to the dose producing noradrenaline depletion. Here also the peak behavioural effect was detected earlier.

4. Pretreatment with reserpine and α-MT had no effect on the intensity of gross behavioural excitation induced by LSD-25 and psilocybin but shortened the duration of the response. The excitation induced by JB-329 was abolished by reserpine pretreatment and was markedly reduced both in intensity and duration by the prior injection of α-MT.

Noradrenaline may be involved in the mode of action of some psychotomimetic drugs. The administration of drugs such as (+)-lysergic acid diethylamide (LSD-25) to both man and animals elicits marked sympathomimetic effects which are considered to be central in origin (Rothlin, 1957; Hollister & Moore, 1967).

While LSD-25 has been regarded as a phenylethylamine capable of stimulating central adrenergic receptors (Brodie, Spector & Shore, 1959; Costa, Gessa, Hirsch, Kuntzman & Brodie, 1962), the drug also lowers brain noradrenaline concentrations (Freedman, 1963), and Siva Sankar, Broer, Cates & Sankar (1964) have suggested that LSD-25 exerts its effects through the release of the amine with the consequential activation of the central sympathetic nervous system.

The present investigation was an attempt to delineate more precisely the role of noradrenaline in behavioural changes induced in rats by LSD-25, psilocybin, which

* Present address: Department of Pharmacology, The University of Texas Southwestern Medical School, Dallas, Texas 75235.

may be regarded as a congener of 5-hydroxytryptamine (5-HT), and JB-329 (Ditran, a mixture of 70% N-ethyl-2-pyrrolidylmethyl cyclopentylphenyl glycolate and 30% N-ethyl-3-piperidyl cyclopentylphenyl glycolate), an atropine-like compound with psychotomimetic activity in man (Abood & Biel, 1962). An attempt was made to determine whether alterations in hypothalamic noradrenaline concentrations could be correlated with changes in behaviour. In addition to observing the effects of the drugs on the gross behaviour of the animal, the drug-induced changes in the acquisition of a conditioned avoidance response were studied. Finally, the effects of the three drugs on the gross behaviour of rats depleted of brain monoamines by the prior administration of reserpine and α-methyl-*p*-tyrosine (α-MT) were investigated.

Some of these results have been communicated to a meeting of the British Pharmacological Society in Nottingham on January 6, 1967.

Methods

Male Wistar rats weighing between 100 and 120 g were used. Drug injections were made by the intraperitoneal route, the time between injection and death or testing being specified in the results.

Estimation of noradrenaline

The rats were killed by a blow on the head and the brain quickly removed. The hypothalamus was regarded as that area defined by Zeman & Innes (1963). The hypothalami from four rats were pooled and weighed. Homogenization in ice-cold 0.4 M perchloric acid was followed by centrifugation (5 min, 0° C, 10,000 *g*). Following extraction (Crout, Creveling & Udenfriend, 1961) from the colourless protein-free supernatant, total catecholamines were assayed by the trihydroxyindole fluorometric procedure of Bertler, Carlsson & Rosengren (1958) and were calculated as μg of noradrenaline (free base) per g of hypothalamic tissue. All values were corrected for a mean recovery of 66.8%.

Each result is the mean ($\pm$S.E.M.) of six observations.

Acquisition of a conditioned avoidance response

Experiments took place in a room totally dependent on artificial light for illumination. In addition, background noise was kept to a minimum.

The apparatus used was a Perspex shuttle box, the two compartments (each 24 cm $\times$ 24 cm $\times$ 23 cm) being separated by a barrier 5 cm high. The floor was an electrified grid of stainless steel rods, 3.5 mm in diameter and 10 mm apart. A constant current shock source delivered the unconditioned stimulus (US) to the grid floor. There was no interval between pulse trains because the mains frequency of 50 c/s was used, the voltage being suitably reduced. The grid floor was not scrambled. A 30 V shock was used in all experiments. Shocks could be delivered to either compartment of the shuttle box. Only one compartment was shocked at a time, the other half of the box acting as the safety area. A buzzer, attached to the shuttle box, supplied the conditioned stimulus (CS). The intensity of sound was 80 db. A positive (correct) response was obtained when the rat crossed from one chamber to the other in response to a buzzing noise of 5 sec duration. A negative

response—failure of the rat to cross the barrier within 5 sec buzzing—was followed by an electric shock.

A naïve rat was placed in either compartment of the shuttle box and allowed 2 min for exploration. This was followed by a period of buzzing (45 sec) so that the animal would not be startled on hearing the CS. After an interval of 75 sec, the CS was applied for 5 sec. If the rat failed to cross the barrier to the safety area, the CS and US were applied together for a further 5 sec. If the rat crossed the barrier within this period, the CS and US were terminated. In the case of rats giving correct responses the time sequence was CS for 5 sec, 55 sec interval, CS for 5 sec, 55 sec interval . . . whereas the sequence for rats exhibiting negative responses was CS for 5 sec, CS and US together for 5 sec, 50 sec interval, CS and US together for 5 sec. . . . A single buzz (CS) of 5 sec duration constituted a trial and the number of trials required for the rat to give nine correct responses within ten consecutive trials was determined. This was termed the 90% acquisition of a conditioned avoidance response criterion (90% criterion). Hence, a rat given a 90% criterion value of 15 (see Table 2) began to make a correct response around the seventh CS-US presentation followed by a further eight successive correct responses.

The schedule of presentation of stimuli was controlled manually and responses were recorded by the operator.

Each result is the mean (±S.E.M.) of ten experiments. Each rat was used once only.

Gross behaviour

The degree of gross behavioural excitation was assessed using the following scoring system: 0, sedation; 1, alert but not moving; 2, rapidly moving around cage; 3, sniffing; 4, licking floor; 5, biting or gnawing (Weissman & Koe, 1965). Groups of five rats were placed in cages (38 cm × 25 cm × 23 cm) immediately after injection and 25 min later the rats were observed for 5 min. The behavioural state of each rat was assessed and a score was assigned to each member of the group. The five scores were totalled (maximum possible score being 25) and this procedure was repeated at 30 min intervals until the behavioural excitation had ceased.

All experiments were done in duplicate and the mean recorded.

The scoring system described above was used by Weissman & Koe (1965) as a means of assessing the degree of behavioural excitation induced in rats by (+)-amphetamine. While it was realized that the gross behavioural excitation caused by the three psychotomimetic drugs under study would not necessarily mimic the (+)-amphetamine-induced excitatory syndrome, it was considered that a quantitative method of assessing the degree of gross behavioural excitation evoked by the three drugs was desirable. The procedure was found to be sufficiently accurate for the needs of the study and was also reproducible. It is of interest to add that scores of 4 and 5 were not recorded during the excitation elicited by the three psychotomimetic drugs. Hence, the maximum score attainable was 15 (Fig. 1b) as opposed to 25 in the case of (+)-amphetamine (Fig. 1a).

Statistics

The order of injecting drug or control was determined by the use of a table of random numbers.

The statistical significance of differences was tested using Student's *t* test.

Drugs and solutions

The following drugs were dissolved in 0.9% w/v sodium chloride solution: LSD-25 (Delysid, Sandoz), psilocybin (Sandoz), JB-329 (Benger Laboratories), 2-bromolysergic acid diethylamide bitartrate (BOL, Koch-Light) and (+)-amphetamine sulphate (Macarthys). α-MT (Merck, Sharp & Dohme) was dissolved in cold distilled water by the addition, drop by drop, of 1 N hydrochloric acid. The pH of the injected solution was 1.6 (Weissman & Koe, 1965). Reserpine (Sigma) was dissolved in 20% w/v ascorbic acid solution. Where applicable, doses reported refer to above salts.

The volume injected ranged from 0.4 to 0.6 ml./100 g body weight. Controls received a similar volume of the solvent used to dissolve the drug.

Results

Effects of psychotomimetic drugs on the noradrenaline content of the rat hypothalamus

The results are summarized in Table 1. LSD-25 1 mg/kg did not significantly lower the noradrenaline content of the rat hypothalamus 1.5 and 3 hr after injection. A significant fall ($P<0.05$) was, however, observed at these times after the administration of 2 mg/kg. The decline reached its maximum of 25% at 3 hr after injection while at 6 hr the noradrenaline level had returned to control values. The amount of psilocybin available permitted determinations only 1.5 and 3 hr after injection. Psilocybin 50 mg/kg had no significant effect at the former time interval but a highly significant fall of 24% ($P<0.001$) was observed at the latter. JB-329 2 mg/kg had no significant effect. Increasing the dose to 4 mg/kg resulted in a significant reduction of 14% ($P<0.05$) 3 hr after injection. In contrast to the three psychotomimetic drugs under investigation, BOL 3 mg/kg had no significant effect at 3 hr on the noradrenaline content of the rat hypothalamus.

TABLE 1. *Effects of LSD-25, psilocybin, JB-329 and BOL on the concentration of noradrenaline in the rat hypothalamus*

Drug	Dose (mg/kg)	Time (hr) between injection and killing	Concentration of noradrenaline (μg/g)		% change from control
			Drug-treated	Control	
LSD-25	1·0	1·5	2·19±0·14	2·41±0·15	− 9·1
LSD-25	1·0	3·0	2·12±0·17	2·33±0·18	− 9·0
LSD-25	2·0	0·5	1·76±0·13	2·00±0·07	−12·0
LSD-25	2·0	1·5	1·76±0·14	2·15±0·05	−18·1*
LSD-25	2·0	3·0	1·52±0·16	2·03±0·11	−25·1*
LSD-25	2·0	6·0	2·22±0·14	2·30±0·17	− 3·5
Psilocybin	50·0	1·5	1·85±0·06	2·03±0·11	− 8·9
Psilocybin	50·0	3·0	1·58±0·08	2·09±0·07	−24·4†
JB-329	2·0	1·5	1·86±0·07	1·89±0·08	− 1·6
JB-329	2·0	3·0	1·81±0·07	1·92±0·08	− 5·7
JB-329	4·0	0·5	1·88±0·07	2·05±0·08	− 8·3
JB-329	4·0	1·5	1·77±0·18	1·99±0·17	−11·1
JB-329	4·0	3·0	1·77±0·10	2·06±0·06	−14·1*
JB-329	4·0	6·0	1·93±0·09	1·91±0·07	+ 1·0
BOL	3·0	1·5	1·59±0·17	1·71±0·16	− 7·0
BOL	3·0	3·0	1·78±0·10	1·89±0·10	− 5·8

All values are the means (±S.E.M.) of six experiments. Significance of differences from control: * $0·05>P>0·01$; † $P<0·001$.

TABLE 2. *Effects of LSD-25, psilocybin and JB-329 on the acquisition of a conditioned avoidance response*

Drug	Dose (mg/kg)	Time (hr) between injection and testing	Trials to 90% criterion		% change from control
			Drug-treated	Control	
LSD-25	0·25	0·5	23·0±2·2	15·1±0·7	+52·3†
LSD-25	0·25	1·5	23·2±2·2	15·6±1·0	+48·7†
LSD-25	0·25	3·0	21·4±2·2	15·5±0·8	+38·1*
LSD-25	0·25	6·0	18·3±1·4	16·5±1·0	+10·9
Psilocybin	25·0	0·5	21·3±2·2	15·7±0·8	+35·7*
Psilocybin	25·0	1·5	22·9±2·3	15·8±0·8	+44·9†
Psilocybin	25·0	3·0	20·6±2·0	16·3±1·0	+26·4
Psilocybin	25·0	6·0	18·1±1·3	16·8±0·8	+ 7·7
JB-329	4·0	0·5	12·3±0·9	16·1±1·1	−23·6*
JB-329	4·0	1·5	12·3±0·6	16·3±0·9	−24·6†
JB-329	4·0	3·0	16·3±1·2	15·2±1·1	+ 7·2
JB-329	4·0	6·0	14·1±0·8	15·0±0·8	− 6·0

All values of the number of trials required to meet the 90% criterion are the means (±S.E.M.) of ten experiments. Significance of differences from control: * $0{\cdot}05>P>0{\cdot}01$; † $0{\cdot}01>P>0{\cdot}001$.

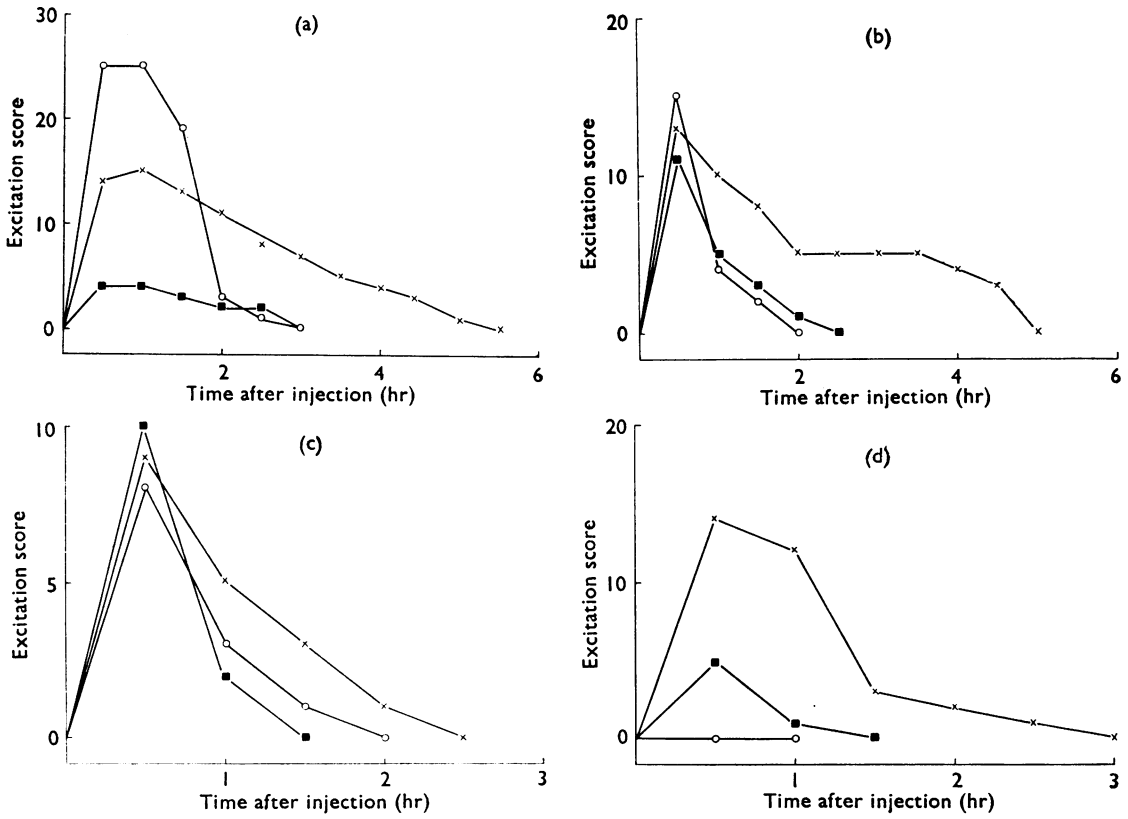

FIG. 1. Effects of reserpine and α-MT on the gross behavioural excitation induced by: (a) (+)-amphetamine 5 mg/kg; (b) LSD-25 2 mg/kg; (c) psilocybin 50 mg/kg, and (d) JB-329 10 mg/kg. Reserpine 5 mg/kg and α-MT 50 mg/kg were injected 18 and 4 hr respectively before the administration of the drug under study. The scoring system of Weissman & Koe (1965) was used, the individual score of each of the five rats in a group was noted and summed every 0.5 hr until excitation had ceased. Each result is the mean of two observations. ×——×, Drug under study alone; ○——○, drug under study+reserpine; ■——■, drug under study + α-MT.

Effects of psychotomimetic drugs on the acquisition of a conditioned avoidance response

The results are summarized in Table 2. LSD-25 0.25 mg/kg significantly increased the number of trials required to meet the 90% criterion at 0.5, 1.5 and 3 hr after injection. A similar picture was obtained with psilocybin 25 mg/kg, acquisition being significantly retarded at 0.5 and 1.5 hr. In contrast to LSD-25 and psilocybin, JB-329 4 mg/kg significantly decreased the number of trials required to meet the 90% criterion at 0.5 and 1.5 hr.

Gross behavioural changes following the administration of psychotomimetic drugs

The effects of the three psychotomimetic drugs were studied on normal rats and on rats depleted of brain monoamines by the administration of reserpine 5 mg/kg and α-MT 50 mg/kg, 18 and 4 hr respectively before the injection of the drug under study. Rats pretreated with reserpine were kept in a room heated to 30° C. (+)-Amphetamine was used as a reference drug.

The results are summarized in Fig. 1. The values for controls are not included because rats pretreated with reserpine and α-MT exhibited a profound and very mild sedation respectively while animals injected with the solvent (0.9 w/v sodium chloride solution) used to dissolve the drugs under study had adopted their normal resting posture by the time the first observation was recorded.

The response to (+)-amphetamine 5 mg/kg reached its maximum 1 hr after injection and the duration of excitation was 5 hr. The prior administration of reserpine enhanced the intensity of the response to (+)-amphetamine but shortened the duration, while pretreatment with α-MT blocked the characteristic excitation induced by the drug. These observations are in agreement with previous findings (Quinton & Halliwell, 1963 ; Weissman & Koe, 1965).

The excitation elicited by LSD-25 2 mg/kg was rapid in onset and lasted 5 hr. Reserpine had no effect on the intensity of the LSD-25-induced excitation but shortened its duration. α-MT slightly decreased the initial response to LSD-25 and also attenuated the duration of excitation. Neither reserpine nor α-MT antagonized the intensity of the excitation following the injection of psilocybin 50 mg/kg, but the duration of the response was reduced.

JB-329, 4 and 10 mg/kg, elicited a rapid and pronounced excitation. Reserpine completely abolished the excitation caused by 10 mg/kg. Pretreatment with α-MT diminished not only the intensity of the response but also shortened the duration of the excitation evoked by 10 mg/kg.

Discussion

The administration of LSD-25, psilocybin and JB-329 caused a slight but significant decrease in the noradrenaline content of the rat hypothalamus.

All three drugs affect the acquisition of a conditioned avoidance response, LSD-25 and psilocybin retarding and JB-329 enhancing the acquisition. A possible explanation for the retarding effect of LSD-25 is that the drug may sensitize the animal to external stimuli (Bradley, 1958), with the result that the rat, in the experimental situation, is flooded with stimuli and, being unable to process the input information adequately, fails to make the correct response (Ray & Bivens, 1966).

There is general agreement in the literature that anticholinergic drugs, as exemplified by atropine and scopolamine, exert an adverse effect on the learning of a conditioned avoidance response while the performance of previously learned behaviour may be enhanced (Longo, 1966 ; Votava, 1967). The finding in this study that JB-329 facilitates the acquisition of such a response in naïve rats was surprising and would suggest that the behavioural excitation induced by JB-329 cannot be attributed to the anticholinergic properties of the drug.

The initial object of this study was to determine whether a correlation exists between the changes in the noradrenaline content of the rat hypothalamus and the alterations in behaviour induced by LSD-25, psilocybin and JB-329. Considering the effect on acquisition in relation to the release of noradrenaline, a comparison of Tables 1 and 2 suggests that no correlation is present. For example, the peak depression of acquisition occurs 0.5 to 1.5 hr after the injection of LSD-25 as against 3 hr for the maximum reduction in noradrenaline levels. Moreover, the dose required to lower amine concentrations is eight times that affecting acquisition. Similarly, a comparison of Table 1 and Fig. 1 indicates that the time of peak gross behavioural excitation induced by LSD-25 does not coincide with that for the maximum fall in noradrenaline content.

The use of monoamine depleting agents such as reserpine and α-MT has been of value in attempts to clarify the mechanism of action of other drugs which evoke excitation, for example (+)-amphetamine. It is currently considered that (+)-amphetamine may owe its behavioural effects to its ability to release newly synthesized noradrenaline (Weissman, Koe & Tenen, 1966 ; Hanson, 1967), for the drug fails to excite animals pretreated with α-MT (Weissman & Koe, 1965). On the other hand, an augmentation of the excitation induced by (+)-amphetamine is observed in reserpinized rats. A consideration of noradrenaline synthesis and storage resolves the apparent paradox whereby the prior administration of two monoamine depleting agents can exert such a dissimilar effect on (+)-amphetamine-induced excitation. Evidence has been presented by Weissman *et al.* (1966) that the anti-amphetamine effect of α-MT is dependent on the ability of the latter to inhibit the enzyme tyrosine hydroxylase and is not related to the degree of noradrenaline depletion. The concept that noradrenaline is present in the peripheral nerve ending in a small functional pool and in a larger storage pool is generally accepted (Axelrod, 1964 ; Kopin, 1966) and Glowinski & Axelrod (1966) have presented evidence for the existence of an analogous situation in the brain. Recent studies suggest that newly synthesized noradrenaline is preferentially taken up by the functional pool (Spector, Gordon, Sjoerdsma & Udenfriend, 1967 ; Kopin, Breese, Krauss & Weise, 1968), so pretreatment with α-MT prevents (+)-amphetamine-induced excitation because there is no newly synthesized noradrenaline available for release. On the other hand, while reserpine produces a marked decrease in brain levels of noradrenaline, by an action presumed to be on storage granules (Carlsson, 1966), not only is noradrenaline synthesis unimpaired (Glowinski, Iversen & Axelrod, 1966) but the brain is partially capable of accumulating the amine within 24 hr of reserpine administration (Glowinski *et al.*, 1966). Consequently, noradrenaline may be available for release by (+)-amphetamine. The behavioural response in reserpinized rats, however, is greater than the excitation evoked in rats solely receiving (+)-amphetamine. Hence, other factors must be involved and the observed enhancement has been the subject of a recent study

(Stolk & Rech, 1967). Among the explanations offered are the possibilities that reserpine pretreatment may sensitize residual noradrenaline stores to the releasing action of (+)-amphetamine and that the latter drug may prevent the destruction of unbound noradrenaline in brain neurones by inhibiting monoamine oxidase. Should (+)-amphetamine inhibit monoamine oxidase in reserpinized animals, as well as release noradrenaline from residual storage sites made more susceptible to release by the alkaloid, the cumulative effect of such actions would be an increase in the effective concentration of the amine in the vicinity of central adrenoceptive receptors and enhanced behavioural excitation could ensue.

Unlike (+)-amphetamine, the ability of LSD-25 and psilocybin to excite rats was not abolished by the prior administration of α-MT. Thus, it seems feasible to postulate that the excitation induced by these agents is not mediated through the release of noradrenaline and that it is more likely to be the result of a direct stimulation of central adrenoceptive receptors. This conclusion would support the hypothesis of Brodie and his associates (Brodie *et al.*, 1959 ; Costa *et al.*, 1962).

Yet LSD-25 and psilocybin lower hypothalamic noradrenaline concentrations and this raises the question as to the significance of this effect in their central actions. The initiation and intensity of excitation produced by the drugs are likely to be due to their direct action on central adrenoceptive receptors, but it is possible that the release of noradrenaline controls the duration of excitation since this is shortened by pretreatment with reserpine and α-MT. On the other hand, the release of noradrenaline may be secondary to the effects of the two drugs on other physiological and biochemical systems present in the brain.

In view of the structural similarity between 5-HT and psilocybin, the possible involvement of 5-HT in the mode of action of LSD-25 and psilocybin cannot be excluded. Indeed, evidence is available indicating that LSD-25 may have a direct action on central 5-HT receptors (Corne & Pickering, 1967 ; Andén, 1968). The sedative effects of reserpine have, however, been attributed to a release of endogenous 5-HT (Costa *et al.*, 1962 ; Brodie, Comer, Costa & Dlabac, 1966), and it is highly improbable that the stimulation of central 5-HT receptors can evoke both excitation and sedation. The question of the possible role of 5-HT is further confused by the finding that the electrically induced release of 5-HT from rat striatal slices is diminished in the presence of LSD-25 (Chase, Breese & Kopin, 1967). Furthermore, the drug decreases brain 5-HT turnover, an observation which has been interpreted as indicating that the release of the amine is prevented by LSD-25 (Diaz, Ngai & Costa, 1968). Diaz *et al.* (1968) also observed an LSD-25-induced reduction in brain noradrenaline and dopamine levels and it was suggested that not only does LSD-25 prevent the release of 5-HT but that the drug also elicits a persistent activation of catecholaminergic neurones. In view of such conflicting evidence it is difficult to define the role of 5-HT in the mode of action of LSD-25 and psilocybin.

In contrast to LSD-25 and psilocybin, the excitation induced by JB-329 was abolished by reserpine pretreatment and was markedly reduced both in intensity and duration by the prior injection of α-MT. These findings may be interpreted as suggesting, in spite of a failure to correlate changes in behaviour with alterations in noradrenaline levels, that the central stimulant actions of JB-329 may depend on the release of noradrenaline because it could be argued that sufficient noradrenaline to cause excitation is no longer available for release from a functional pool in rats pretreated with the monoamine depleting agents. A possible argument against the

hypothesis that JB-329 may owe its activity to a release of noradrenaline is the observed difference in the response of reserpinized rats to (+)-amphetamine and JB-329. A conceivable explanation is that JB-329 has no effect on the storage, release and inactivation of noradrenaline other than an ability to release the amine from a functional pool. The amount of noradrenaline available for release from such a pool may be subnormal in the reserpinized rat and, while JB-329 may release the amine, the amount released is insufficient to excite the animal. A possible reason for the enhanced excitation induced by (+)-amphetamine in rats pretreated with reserpine was offered above and it is suggested that the reason for the observed behavioural difference in the activity of JB-329 and (+)-amphetamine in reserpinized rats may be explained in terms of the effective amount of the amine available for interaction with central adrenoceptive receptors. Obviously, the validity of these assumptions cannot be resolved until the full spectrum of (+)-amphetamine activity on noradrenergic systems is completely clarified. The failure to correlate alterations in hypothalamic noradrenaline content with changes in behaviour does not necessarily invalidate the postulate that the central stimulant actions of JB-329 may depend upon the release of noradrenaline, since the possibility cannot be excluded that the JB-329-induced excitation depends on the release of the amine from a functional pool and that the measurement of total noradrenaline content does not give a valid reflection of this phenomenon.

Although the results of the conditioned avoidance response experiments suggest that the cholinolytic actions of JB-329 are not primarily responsible for the behavioural excitation evoked by the drug, the possibility of this pharmacological property contributing to the mode of action of the drug cannot be excluded, especially in view of Carlton's (1963) attractive hypothesis which suggests that there are two interacting systems in the brain: an inhibitory cholinergic system which exerts its actions normally by inhibiting an activating system which, as the name implies, normally activates behaviour. The activating system is assumed to be adrenergic, so the administration of an anticholinergic drug can have no effect in an animal depleted of brain adrenergic amines. Such a hypothesis could explain the lack of effect of JB-329 in rats pretreated with reserpine and α-MT. The results from the conditioned avoidance response experiments, however, together with the fact that the same dose of JB-329 affects both behaviour and noradrenaline concentrations, imply that release of noradrenaline rather than cholinolytic activity may be involved in the central excitation elicited by the drug.

Finally, the similarity in the pharmacological profiles of LSD-25 and psilocybin observed in this study supports the suggestion, based on cross-tolerance studies in man, that the two drugs may have the same mechanism of action (Isbell, Wolbach, Wikler & Miner, 1961), while the tentative suggestion that JB-329 may excite rats through the release of noradrenaline supports the hypothesis that an adrenergic component may be involved in the mode of action of the drug in man (Neubauer, Sundland & Gershon, 1966).

The excellent technical assistance of Mr. D. White is gratefully acknowledged. Thanks are also extended to Drs. P. A. Shore, A. Giachetti, and T. C. Muir for their criticisms of the manuscript.

REFERENCES

ABOOD, L. G. & BIEL, J. H. (1962). Anticholinergic psychotomimetic agents. *Int. Rev. Neurobiol.*, **4**, 217–273.

ANDÉN, N.-E. (1968). Discussion of serotonin and dopamine in the extra-pyramidal system. *Adv. Pharmac.*, **6A**, 347–349.

AXELROD, J. (1964). The uptake and release of catecholamines and the effect of drugs. *Prog. Brain Res.*, **8**, 81–89.

BERTLER, Å., CARLSSON, A. & ROSENGREN, E. (1958). A method for the fluorimetric determination of adrenaline and noradrenaline in tissues. *Acta physiol. scand.*, **44**, 273–292.

BRADLEY, P. B. (1958). *Reticular Formation of the Brain*, ed. Jasper, H. H., Proctor, L. D., Knighton, R. S., Noshay, W. C. & Costello, R. T., pp. 123–149. London: Churchill.

BRODIE, B. B., COMER, M. S., COSTA, E. & DLABAC, A. (1966). The role of brain serotonin in the mechanism of the central action of reserpine. *J. Pharmac. exp. Ther.*, **152**, 340–349.

BRODIE, B. B., SPECTOR, S. & SHORE, P. A. (1959). Interaction of drugs with norepinephrine in the brain. *Pharmac. Rev.*, **11**, 548–564.

CARLSSON, A. (1966). *5-Hydroxytryptamine and Related Indolealkylamines*, ed. Erspamer, V., pp. 529–592. Berlin: Springer-Verlag.

CARLTON, P. L. (1963). Cholinergic mechanisms in the control of behavior by the brain. *Psychol. Rev.*, **70**, 19–39.

CHASE, T. N., BREESE, G. R. & KOPIN, I. J. (1967). Serotonin release from brain slices by electrical stimulation: regional differences and effect of LSD. *Science, N.Y.*, **157**, 1461–1463.

COSTA, E., GESSA, G. L., HIRSCH, C., KUNTZMAN, R. & BRODIE, B. B. (1962). On current status of serotonin as a brain neurohormone and in action of reserpinelike drugs. *Ann. N.Y. Acad. Sci.*, **96**, 118–131.

CORNE, S. J. & PICKERING, R. W. (1967). A possible correlation between drug-induced hallucinations in man and a behavioural response in mice. *Psychopharmacologia*, **11**, 65–78.

CROUT, J. R., CREVELING, C. R. & UDENFRIEND, S. (1961). Norepinephrine metabolism in rat brain and heart. *J. Pharmac. exp. Ther.*, **132**, 269–277.

DIAZ, P. M., NGAI, S. H. & COSTA, E. (1968). Factors modulating brain serotonin turnover. *Adv. Pharmac.*, **6B**, 75–92.

FREEDMAN, D. X. (1963). Psychotomimetic drugs and brain biogenic amines. *Am. J. Psychiat.*, **119**, 843–850.

GLOWINSKI, J. & AXELROD, J. (1966). Effects of drugs on the disposition of H^3-norepinephrine in the rat brain. *Pharmac. Rev.*, **18**, 775–785.

GLOWINSKI, J., IVERSEN, L. L. & AXELROD, J. (1966). Storage and synthesis of norepinephrine in the reserpine-treated rat brain. *J. Pharmac. exp. Ther.*, **151**, 385–399.

HANSON, L. C. F. (1967). Evidence that the central action of (+)-amphetamine is mediated via catecholamines. *Psychopharmacologia*, **10**, 289–297.

HOLLISTER, L. E. & MOORE, F. (1967). Urinary catecholamine excretion following lysergic acid diethylamide in man. *Psychopharmacologia*, **11**, 270–275.

ISBELL, H., WOLBACH, A. B., WIKLER, A. & MINER, E. J. (1961). Cross tolerance between LSD and psilocybin. *Psychopharmacologia*, **2**, 147–159.

KOPIN, I. J. (1966). Biochemical aspects of release of norepinephrine and other amines from sympathetic nerve endings. *Pharmac. Rev.*, **18**, 513–523.

KOPIN, I. J., BREESE, G. R., KRAUSS, K. R. & WEISE, V. K. (1968). Selective release of newly synthesized norepinephrine from the cat spleen during sympathetic nerve stimulation. *J. Pharmac. exp. Ther.*, **161**, 271–278.

LONGO, V. G. (1966). Behavioral and electroencephalographic effects of atropine and related compounds. *Pharmac. Rev.*, **18**, 965–996.

NEUBAUER, H., SUNDLAND, D. & GERSHON, S. (1966). Ditran and its antagonists in a mixed psychiatric population. *J. nerv. ment. Dis.*, **142**, 265–277.

QUINTON, R. M. & HALLIWELL, G. (1963). Effects of α-methyl DOPA and DOPA on the amphetamine excitatory response in reserpinized rats. *Nature, Lond.*, **200**, 178–179.

RAY, O. S. & BIVENS, L. W. (1966). Performance as a function of drug, dose, and level of training. *Psychopharmacologia*, **10**, 103–109.

ROTHLIN, E. (1957). *Psychotropic Drugs*, ed. Garattini, S. & Ghetti, V., pp. 36–47. Amsterdam: Elsevier.

SIVA SANKAR, D. V., BROER, H. H., CATES, N. & SANKAR, D. B. (1964). Studies on biogenic amines and psychoactive drug actions, with special reference to lysergic acid diethylamide. *Trans. N.Y. Acad. Sci.*, **26**, 369–376.

SPECTOR, S., GORDON, R., SJOERDSMA, A. & UDENFRIEND, S. (1967). End-product inhibition of tyrosine hydroxylase as a possible mechanism for regulation of norepinephrine synthesis. *Molec. Pharmac.*, **3**, 549–555.

STOLK, J. M. & RECH, R. H. (1967). Enhanced stimulant effects of *d*-amphetamine on the spontaneous locomotor activity of rats treated with reserpine. *J. Pharmac. exp. Ther.*, **158**, 140–149.

VOTAVA, Z. (1967). Pharmacology of central cholinergic synapses. *A. Rev. Pharmac.*, **7**, 223–240.

WEISSMAN, A. & KOE, B. K. (1965). Behavioural effects of L-α-methyltyrosine, an inhibitor of tyrosine hydroxylase. *Life Sci., Oxford*, **4**, 1037–1048.

WEISSMAN, A., KOE, B. K. & TENEN, S. S. (1966). Antiamphetamine effects following inhibition of tyrosine hydroxylase. *J. Pharmac. exp. Ther.*, **151**, 339–352.

ZEMAN, W. & INNES, J. R. M. (1963). *Craigie's Neuroanatomy of the Rat*, pp. 23 and 119. New York: Academic Press.

(*Received May* 10, 1968)