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HEAD TWITCHES INDUCED BY LSD AND QUIPAZINE: SIMILARITIES AND DIFFERENCES

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Summary Intraperitoneal injections of LSD and quipazine produced head twitches in the rat. The dose-response curves were bell-shaped, with maxima at 50 μg/kg for LSD and 5 mg/kg for quipazine. In a single animal the responses to two consecutive treatments with the same agent were similar, and the correlation between the responsiveness to LSD and quipazine was high ($r = 0.86$). Head twitches induced by LSD were inhibited more strongly than those observed after quipazine by drugs impairing noradrenergic transmission and by morphine. The converse was true for a serotonin antagonist, cyproheptadine, and for morphine derivatives, particularly *N*-cyclopropylmethylnorazidomorphine. The results suggest an involvement of the central noradrenergic system in the response to LSD in addition to a serotonergic mechanism, and auxillary participation of one kind of opiate receptors in the action of quipazine.

Rapid oscillatory head movements called "head twitches" (Keller and Umbreit, 1956), were regarded as a measure of stimulation of central serotonin receptors (Bedard and Pycock, 1977; Corne, Pickering and Warner, 1963), as an animal behaviour correlate of hallucinations in man (Corne and Pickering, 1967) and as a part of so-called quasi-abstinence syndrome (Collier, 1965).

Among the many compounds capable of eliciting head twitches are LSD (Bedard and Pycock, 1977; Corne and Pickering, 1967; Keller and Umbreit, 1956) and quipazine (Bedard and Pycock, 1977; Malick, Doren and Barnett, 1977). Both drugs are believed to stimulate central serotonin receptors (Aghajanian, 1972; Hong, Sancilio, Vargas and Pardo, 1969; Rodriguez, Rojas-Ramirez and Drucker-Colin, 1973).

Recently, Bedard and Pycock (1977) have reported that head twitches induced by 5-hydroxytryptophan were not changed by drugs interfering with either cholinergic or noradrenergic systems in the brain. This finding was at variance with the results of Maj, Baran, Bigajska, Rogóz and Skuza (1978), who have found that head twitches induced in rats and mice by 5-hydroxytryptophan were inhibited by phenoxybenzamine, phentolamine and subcataleptic doses of 18 out of 19 neuroleptics tested (spiperone was practically equipotent in inducing catalepsy and inhibiting head twitch response). Also, the finding that clonidine did not affect the head twitch response contradicted previous results from this laboratory that low doses of clonidine effectively inhibited the head twitches brought about by 5-hydroxytryptophan or 5-methoxytryptamine (Bednarczyk and Vetulani, 1978) as well as "wet dog shakes" in the course of morphine withdrawal (Bednarczyk and Vetulani, 1978; Vetulani and Bednarczyk, 1977). In an extension of these studies the effect of agents impairing noradrenergic transmission, clonidine and phenoxybenzamine have been examined on head twitches produced by LSD and quipazine. The effect of opiates, morphine and two novel morphine derivatives: azidomorphine and *N*-cyclopropylmethylnorazidomorphine (CAM) were also tested. While azidomorphine was reported to be a potent analgesic producing only minimal tolerance (Knoll, 1973), CAM is an antagonist of morphine analgesia but mimicks the action of morphine in some *in vitro* preparations, e.g. in the isolated guinea-pig ileum (Knoll, 1977). On these grounds Knoll (1977) assumed that there are two kinds of opiate receptors, A and B: A receptors are those stimulated and B receptors are those inhibited by CAM. The present authors have shown that A receptors are involved in the phenomena of opiate dependence and abstinence (Vetulani, Bednarczyk and Reichenberg, 1978); consequently, it was concluded that azidomorphine stimulates predominantly, if not exclusively, the opiate B receptors.

The present results show that not only a serotonin receptor blocking agent, cyproheptadine (Cline-schmidt and Lotti, 1974), but also clonidine, phenoxybenzamine and opiates inhibit the shaking behaviour induced by LSD and quipazine.

METHODS

Animals

Male Wistar rats, 170-210 g, fed *ad libitum* with a standard pellet food (Murigran) and having free access to water, were used throughout. They were kept in colony cages until the experiment. The rats used in iterative tests were marked by ear cuts.

Drugs

LSD (lysergic acid diethylamide hydrobromide; Spofa), quipazine hydrochloride (Miles Labs), phenoxybenzamine hydrochloride ("Dibenyline"; Smith, Kline & French), clonidine hydrochloride (Boehringer, Sohn), cyproheptadine hydrochloride (isolated from tablets, "Periactin"; Merck, Sharp & Dohme), morphine hydrochloride (Polfa), azidomorphine and CAM (*N*-cyclopropylmethylnorazidomorphine) were synthesized in the Department of Organic Chemistry, Kossuth Lajos University, Debrecen, Hungary. All drugs were injected intraperitoneally, as solutions in saline. The doses refer to the form listed above.

Shaking behaviour

The number of head twitches was counted for 15 min starting immediately after an injection of LSD, and for 40 min (if not stated to the contrary) after quipazine administration. For observations the rats were placed in 21 × 21 × 22 cm wire cages. The drugs tested for antagonistic action were given at intervals reported in Table 1.

Variability coefficient was defined as $V = SD:\bar{X}$ (where SD—standard deviation; $\bar{X}$—arithmetical mean); correlation coefficients r and ID_{50} values (defined as doses reducing the head twitch frequency by 50%) were calculated with an HP-65 programmable calculator; for calculation of ID_{50} values the formulae derived by Litchfield and Wilcoxon (1949) were used.

RESULTS

1. *Variability of responses and their repeatability*

High variability of responses, both within groups tested on the same day and among groups tested in various days, was noted. The variability coefficients V, calculated for the combined control groups, were 0.80 (N = 136) for 0.1 mg/kg LSD; and 0.65 (N = 78) for 5 mg/kg quipazine. However, the responses were stable for individual animals: the responses to the first and the subsequent treatment (1 week apart) with the same agent were highly correlated, the correlation coefficient r = 0.91 (N = 8) for 0.1 mg/kg LSD, and 0.87 (N = 7) for 10 mg/kg quipazine.

2. *Cross-correlation of responses to LSD and quipazine*

Although the frequency of head twitches produced by quipazine was higher, and the effect of the drug more prolonged than that of LSD, the correlation coefficient for the responses to subsequent treatment of the same rat with another drug was very high. In two experiments, in one of which the rat received firstly 10 mg/kg quipazine followed 24 hr later by 0.1 mg/kg LSD, and in the second the schedule was reversed, the coefficient r for the first and second response was for each case 0.86 (N = 8). In other, unpublished experiments no correlation between the response to LSD and 5-hydroxytryptophan or 5-methoxytryptamine was found.

3. *Dose-response relationship*

Both LSD and quipazine produced head twitches only when given in a certain dose range. The dose-response curves were bell-shaped (Fig. 1), indicating an autoinhibitory effect of large doses.

4. *The effects of agents affecting monoaminergic transmission or acting on opiate receptors (Table 1)*

All agents tested inhibited the head twitches produced by LSD and quipazine. The drugs impairing noradrenergic transmission, phenoxybenzamine and clonidine, were more potent in inhibiting the response to LSD; similarly morphine was more potent in its action against LSD than against quipazine. On the other hand, cyproheptadine, and also azidomorphine derivatives, particularly CAM, were preferential antagonists of quipazine-induced head twitches. The action of azidomorphine was short-lasting, and therefore its effect was observed only during 15 min following the administration of the drug.

Table 1. Inhibition by drugs of head twitch response produced by LSD and quipazine

Drug	LSD, 100 μg/kg		Quipazine, 5 mg/kg	
	ID_{50} (μg/kg)	S	ID_{50} (μg/kg)	S
PBZ	1500 (600–3500)	0.20 (0.05–0.78)	Non-monotonous relationship†	
CLO	5 (2–16)	0.18 (0.05–0.67)	43 (22–83)	0.27 (0.10–0.78)
CHD	180 (30–1160)	0.19 (0.03–1.12)	14 (7–28)	0.36 (0.13–0.95)
MOR	1380 (950–1990)*	0.45 (0.31–0.66)	3040 (2350–3920)*	0.64 (0.45–0.86)
CAM	540 (390–740)*	0.52 (0.33–0.82)	3 (0.6–18)*	0.07 (0.01–0.90)
AZM	83 (70–98)	0.63 (0.52–0.75)	44 (29–67)	0.32 (0.06–1.83)

PBZ—Phenoxybenzamine, given 90 min before LSD or quipazine; CLO–clonidine, 60 min; CHD—cyproheptadine, 30 min; MOR—morphine, 30 min; CAM-*N*-cyclopropylmethylnorazidomorphine, 30 min; AZM—azidomorphine, given simultaneously with LSD or 15 min after quipazine injection.

The number of head twitches was counted for 15 min after LSD and for 40 min after quipazine, with the exception of the experiment with AZM, in which the number of twitches was counted for 15 min immediately after AZM injection.

* These data were published earlier in a short communication (Vetulani *et al.*, 1978).

† See Fig. 2.

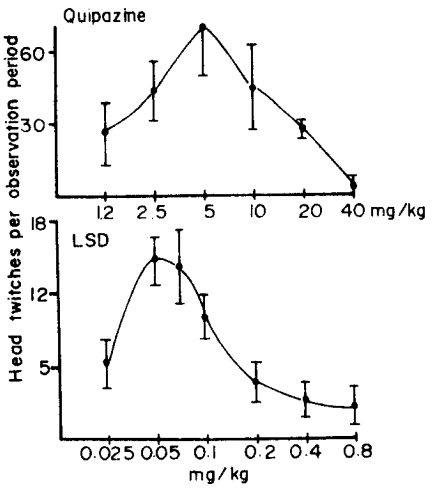

Fig. 1. Dose-effect relationship in the action of LSD and quipazine. Ordinate: Number of head twitches per observation period (15 min for LSD, 40 min for quipazine). Abscissa: dose in mg/kg. Observations began immediately after the administration of the drug. Each point is the mean ± SEM of at least 6 results.

Phenoxybenzamine produced a biphasic inhibition of the head twitches produced by quipazine (Fig. 2), and therefore it was impossible to calculate its meaningful ID_{50} value.

DISCUSSION

The results presented here indicate that head twitches produced by LSD and quipazine may have in common a large part of their mechanism of action, although there are also some differences between the twitches produced by them. In spite of individual variability of the response, the correlation between the responses to successive treatments with two different agents is high, and similar to that observed if the same agent was used twice. On the other hand, it has been found that no correlation exists between the action of LSD and that of 5-hydroxytryptophan or 5-methoxytryptamine (Bednarczyk, 1978), and therefore the similarity in the response to LSD and quipazine cannot be due merely to the involvement of serotonin neurones in the phenomenon observed. In contrast to 5-hydroxytryptophan, both drugs in higher doses display self-inhibitory properties, as had been earlier described by Bedard and Pycock (1977) and Corne and Pickering (1967); large doses of LSD were also reported to inhibit head twitches induced by 5-hydroxytryptophan (Cowan and Watson, 1978; Reichenberg, Wiszniowska and Marchaj, 1975) and it was found that large doses of quipazine exert a similar effect (Cowan and Watson, 1978). It should be also noted that rats respond similarly when quipazine is substituted for LSD when the latter is used as a discriminative stimulus, while other serotonin agonists are perceived as different cues (Kuhn, White and Appel, 1978).

The opinion that serotonin is involved in head-twitch behaviour brought about by LSD and quipazine is at present indisputable, particularly as cyproheptadine, a compound believed to block serotonin receptors preferentially (Clineschmidt and Lotti, 1974), inhibits this behaviour. The noradrenergic system, however, also seems to be involved, as the head twitch response to LSD and quipazine, as was that to 5-hydrotytryptophan (Maj *et al.*, 1978; Bednarczyk and Vetulani, 1978) was blocked by phenoxybenzamine and clonidine. The latter drug seems to behave in this respect as an alpha-adrenergic antagonist and studies both in brain tissue (Skolnick and Daly, 1975; Vetulani, Leith, Stawarz and Sulser, 1977) and in blood platelets (Jakobs, 1978) indicate the existence of a specific type of a post-synaptic alpha-adrenoceptor that is stimulated only by endogenous catecholamines, but is inhibited by clonidine and other synthetic alpha-adrenomimetics. Head twitches produced by LSD and quipazine may also depend on stimulation of this particular alpha-adrenoceptor.

The difference between the action of LSD and quipazine may be seen when the relative potencies of antagonists of various groups are compared. Thus, a serotinin antagonist, cyproheptadine, is approx. 13 times more potent in inhibiting quipazine- than LSD-induced head twitches. Conversely, the drugs inhibiting neurotransmission at adrenergic neurones seem to act preferentially on LSD-induced head twitches. This is particularly true for clonidine, which is extremely potent in its action against the response to LSD, while its potency against quipazine is similar to that against 5-hydroxytryptophan (Bednarczyk and

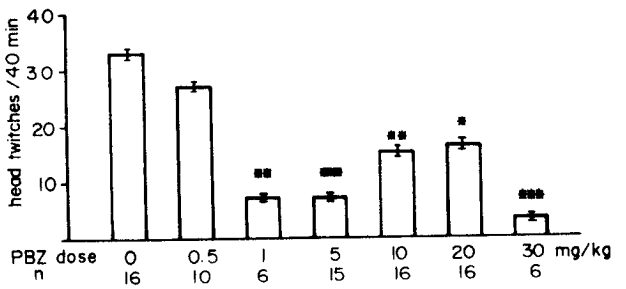

Fig. 2. The effect of phenoxybenzamine on quipazine-induced head twitches. Phenoxybenzamine (PBZ) was given 90 min before 5 mg/kg quipazine, n = number of rats tested.
$^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ (significance of the difference from the controls, t-test).

Vetualni, 1978). These data suggest that a serotonergic mechanism plays a more decisive role in the action of quipazine, while the central adrenergic system is to some extent involved in the LSD-induced head twitches.

Various opiates also differ in their potency as inhibitors of head twitches induced by LSD and quipazine. Morphine, which inhibits the head twitches produced by 5-hydroxytryptophan (Corne *et al.*, 1963), is more potent in inhibiting the syndrome induced by LSD, while azido derivatives of morphine are more effective in prevention of quipazine shaking behaviour.

The potent inhibitor of shaking behaviour accompanying morphine abstinence CAM (Vetulani *et al.*, 1978) inhibited the head twitches produced by LSD in doses not significantly different from the ID_{50} for pinna reflex, which was 0.94 (0.59–1.49) mg/kg (Bednarczyk, 1978). Conversely, it was extremely potent in inhibiting the head twitches induced by quipazine. Its ID_{50} in this test was almost 30 times lower than the ID_{50} for shaking in the course of spontaneous morphine withdrawal (Vetulani *et al.*, 1978), and 180 times lower than the ID_{50} for LSD head twitches.

The present data indicate that opiate receptors of the A type may be involved specifically in the head twitch response to quipazine, while it is possible that opiate B receptors also play a role in the response to LSD. The latter suggestion is in agreement with the data of Knoll (1977), indicating that opiate B receptors are involved in the presynaptic control of noradrenaline release; as shown here noradrenergic transmission is important for the expression of head twitch behaviour after LSD.

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