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INTERACTIONS BETWEEN EPINEPHRINE AND SOME PSYCHOTOMIMETIC DRUGS[1]

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This paper describes the potentiation of the epinephrine effect on the nictitating membrane in cats by a number of psychotomimetics and chemically related drugs. The following facts suggested this investigation: 1) in rabbits, central sympathetic excitation follows the injection of LSD-25 (*d*-lysergic acid diethylamide) but not that of BOL-148 (2-brom-LSD) (Rothlin, 1957a); BOL-148, in contrast to LSD-25, is probably devoid of psychotomimetic effects (Snow *et al.*, 1955); 2) in rats 10 μg/kg of LSD-25, i.p., potentiate the adrenal ascorbic acid depletion whereas 40 μg/kg of BOL-148, i.p., are inactive (Costa and Zetler, 1958); 3) in man the pupillary dilatation caused by LSD is probably the result of central sympathetic stimulation (Poloni and Maffezoni, 1952; Sokoloff *et al.*, 1957); 4) serotonin (Reid and Rand, 1951) and probably bufotenine (Raymond-Hamet, 1942, 1943) release epinephrine from the adrenal medulla. It is not known whether bufotenine causes hypersensitivity of the nictitating membrane of the cat to injected epinephrine as does serotonin (Lecomte, 1955). In contrast to serotonin, bufotenine evokes hallucinatory experiences in man (Fabing and Hawkins, 1956.).

METHODS. Cats of 2 to 3 kg body weight were used. Under ether anesthesia the femoral vein was cannulated and 40 mg/kg of chloralose plus 15 mg/kg of sodium pentobarbital in saline solution were injected. Additional barbiturate was administered as necessary.

Respiration rate, blood pressure and nictitating membrane contractions were recorded by means of a Sanborn Polyviso oscillograph. The respiratory excursions were recorded by a piezoelectric transducer tied to the abdominal wall. Blood pressure was measured from a cannulated branch of the femoral artery connected to a Sanborn electromanometer. Contractions of the nictitating membrane were recorded by attaching it by a thread to a rubber diaphragm covering a research tambour 13 mm in diameter. The tambour was filled with distilled water and connected through a continuous hydraulic system to a second Sanborn electromanometer. The plot of diaphragm tension (50 to 400 mg) against pen excursions was linear. Diaphragm movement during recording was barely detectable visually. The system, therefore, may be considered fairly isometric.

Eighty-four cats were used in this study. In fourteen of these animals the right superior sympathetic ganglion had been removed 7 to 9 days preceding the experiment.

All drugs were dissolved in physiological saline and injected into the femoral vein over a period of 5 seconds. Epinephrine and norepinephrine were used in the form of their *levo* isomers. Small amounts of ascorbic acid were added to solutions of these amines in order to prevent oxidation. The time interval between 2 consecutive injections was 2 minutes. The mean threshold doses were determined as described in detail under RESULTS. The arithmetical means, standard errors, and P values were computed applying conventional formulae and student's "t" test.

The following drugs were tested for their capacity to increase the contractile response of the nictitating membrane to epinephrine: 5-hydroxytryptamine creatinine sulfate (serotonin); tryptamine HCl; 5-hydroxy-N,N-dimethyltryptamine monoxalate (bufotenine); 1-benzyl-2-methyl-5-methoxytryptamine HCl (1-benzyl-2,5-dimethylserotonin, BAS); 1-benzyl-2-methyl-5-methoxy-N,N-dimethyltryptamine HCl (1-benzyl-2,5-dimethylbufotenine, BAB); mescaline hydrochloride; harmine HCl; Dibenamine HCl (N,N-dibenzyl-β-chloroethylamine HCl); cocaine HCl; LSD-25; BOL-148; ALD-52 (1-acetyl-lysergic acid diethylamine-bitartrate); LAE-32 (*d*-lysergic

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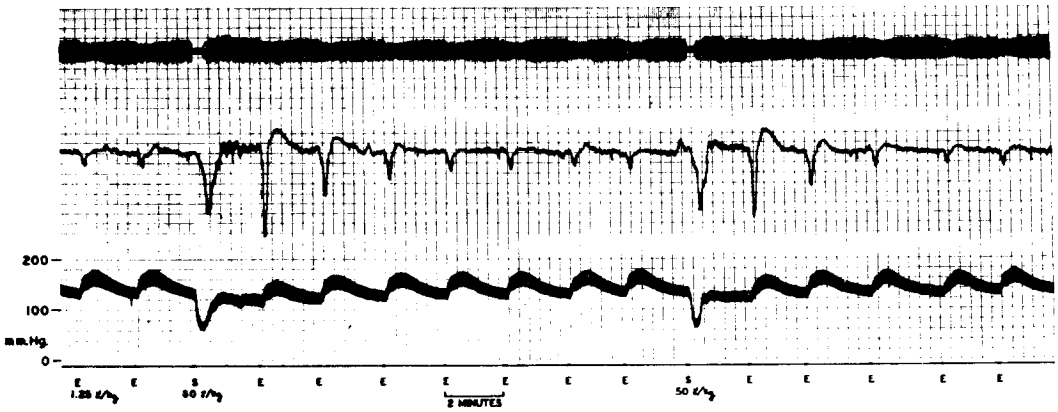


FIG. 1. The threshold dose of serotonin (80 μg/kg) for epinephrine potentiation on nictitating membrane is greater than that causing a contraction.
Cat (3 kg) anesthetized with sodium pentobarbital (15 mg/kg, i.v.) and chloralose (40 mg/kg, i.v.). From top to bottom: Respiratory movement, normal nictitating membrane (downward excursion means contraction), blood pressure. E = epinephrine; S = serotonin.

acid ethylamide bimaleate); ergotamine tartrate; DHE (dihydroergotamine methanesulfonate); Methergine (methylergonovine tartrate); LSM (*d*-lysergic acid morpholide)[3].

RESULTS. *Indolalkylamines.* Following serotonin injections (10 to 15 μg/kg, i.v.) a biphasic blood pressure response (hypotension succeeded by a delayed, slight hypertension) was noted as well as a contraction of the nictitating membrane. As can be seen from figure 1, larger doses (80 and 50 μg/kg, i.v.) caused a temporary respiratory arrest.

The bufotenine mean threshold dose (44 μg/kg, i.v.) causing the nictitating membrane to contract was greater than the one inducing hypotension, whereas with serotonin a significant difference in these two thresholds was not observed. In contrast to serotonin, a dose of bufotenine greater than 100 μg/kg frequently elicited a hypertensive response. This hypertension was diminished or abolished by adrenalectomy or by pretreatment with Regitine (phentolamine HCl) (1 mg/kg, i.v.). Premedication with atropine sulfate (200 to 300 μg/kg, i.v.) usually reversed the hypotension induced by bufotenine, 30 to 100 μg/kg then being sufficient to cause a hypertensive response.

[3] It is our pleasure to acknowledge our indebtedness to the following pharmaceutical companies: Sandoz, Hanover, N. J., for the generous supply of LSD-25 and its analogues; Eli Lilly, Indianapolis, Indiana, for LSM; E. R. Squibb, New York, for BAS; and Merck, Sharp & Dohme, West Point, Pa., for BAB.

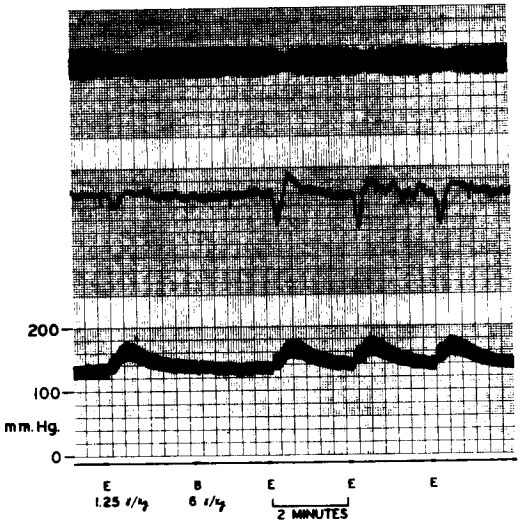


FIG. 2. An epinephrine potentiating threshold dose of bufotenine does not cause a contraction of the normal nictitating membrane.
Cat (2.8 kg) anesthetized with sodium pentobarbital (15 mg/kg, i.v.); legend as in fig. 1; B = bufotenine.

As can be seen from figures 1 and 2, both bufotenine and serotonin increased the effect of epinephrine on the nictitating membrane. The reasons previously mentioned prompted us to perform a quantitative analysis of the epinephrine potentiating effect of bufotenine and serotonin. The epinephrine potentiation by serotonin had already been investigated by Lecomte (1955). Trendelenburg (1956) demonstrated that serot-

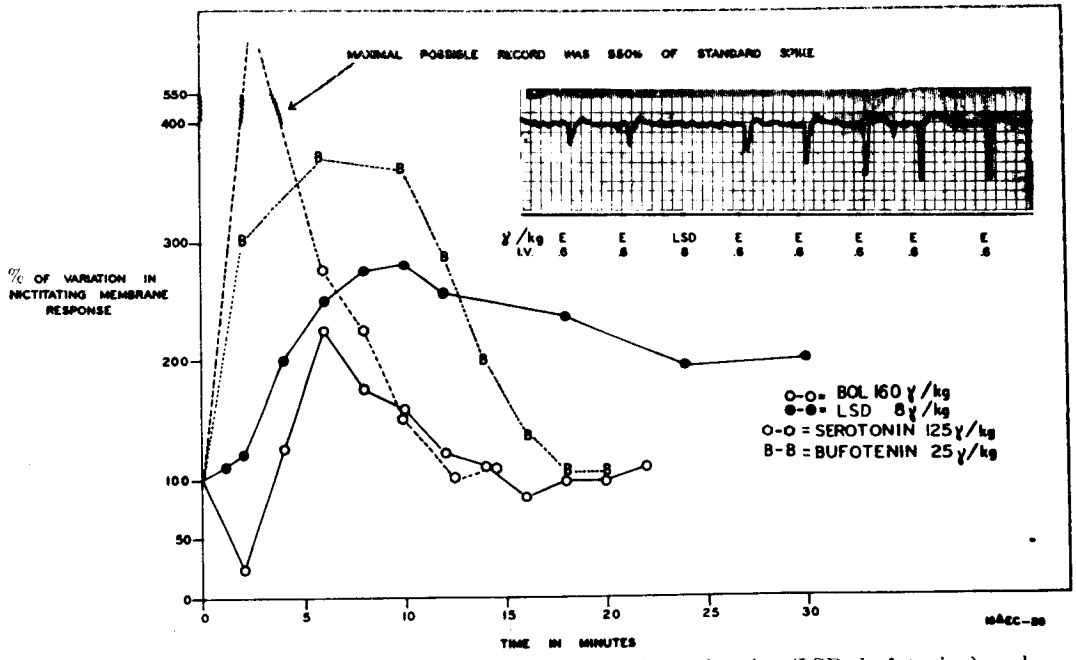


FIG. 3. The epinephrine potentiation evoked by two psychotomimetics (LSD, bufotenine) endures longer than that of larger doses of the respective chemical analogues (BOL, serotonin) devoid of psychotomimetic effect.

The data reported were obtained with the same cat (2.7 kg), anesthetized as in fig. 1. Ordinate: 100 = epinephrine response before medications.

onin potentiates the nictitating membrane responses to submaximal preganglionic stimulation.

Figure 3 summarizes the results obtained in one experiment in which the potentiating effects of LSD and bufotenine were compared to those of BOL and serotonin in the same cat. A small dose of bufotenine (25 μg/kg) potentiated the response of the nictitating membrane to epinephrine for a period of 15 minutes. The potentiating effect of 125 μg/kg of serotonin, although more pronounced, was more fleeting than that of bufotenine. From the data presented in figure 3 it appears obvious that the duration of the potentiation is an important characteristic of this drug action. This also can be emphasized by comparing (figure 3) the effect of LSD-25 (8 μg/kg) to that of BOL-148 (160 μg/kg). The drugs causing epinephrine potentiation were screened by determining the mean threshold doses evoking a hypersensitivity to injected epinephrine. The minimal quantity of a drug (μg/kg, i.v.) causing at least a doubling of the height of the response of the nictitating membrane to a threshold dose of epinephrine injected intravenously every 2 minutes was considered the threshold potentiating dose. Our testing scheme required, furthermore, that the increased response to epinephrine should occur 3 times during 10 minutes.

The mean threshold potentiating doses of both bufotenine and serotonin for normal and denervated nictitating membranes are presented in table 1. In the table are also listed the mean threshold doses of epinephrine, serotonin and bufotenine causing a contraction of the same nictitating membranes used for the screening of the potentiating effect. In normal as well as denervated membranes epinephrine was more active than both bufotenine ($P < 0.001$) and serotonin ($P < 0.001$) in evoking contractions of the nictitating membrane. After denervation the threshold contracting doses of 3 drugs were significantly decreased. Although in each experiment serotonin was a more effective stimulating agent than bufotenine, the difference between the two means is statistically significant only for denervated membranes. In normal nictitating membranes the bufotenine threshold potentiating dose was smaller than the contracting one ($P < 0.01$), whereas the potentiating threshold dose of serotonin was greater than the contract-

TABLE 1

Actions and interactions of epinephrine, serotonin and bufotenine on normal and denervated nictitating membrane

		Normal Membrane* n = 10		Denervated Membrane† n = 6		P
		μg/kg	S.E.	μg/kg	S.E.	
Threshold doses evoking contractions	Epinephrine	0.74	±0.14	0.07	±0.009	0.001
	Serotonin	22.40	±6.27	2.0	±0.516	0.01
	Bufotenine	44.30	±9.05	5.5	±1.430	0.001
Threshold doses potentiating epinephrine response	Serotonin	114.00	±23.96	25.8	±5.040	0.01
	Bufotenine	5.50	±0.89	8.3	±2.540	0.9

* Normal nictitating membrane: stimulating threshold dose (th.d.), P = 0.05; potentiating th.d., P = 0.001 (differences between serotonin and bufotenine).

† Denervated nictitating membrane: stimulating th.d., P = 0.05; potentiating th.d., P = 0.02 (differences between serotonin and bufotenine).

TABLE 2

Threshold doses (μg/kg) potentiating epinephrine response

	Normal Nictitating Membrane			Denervated Nictitating Membrane			P
	No. of animals	Mean	S.E.	No. of animals	Mean	S.E.	
Mescaline	5	152.0	±15.94	—	—	—	
Harmine	5	58.0	±18.07	—	—	—	
LSD-25	6	1.42	±0.57	4	1.14	±0.38	0.6
Dibenamine	4	2.0	±0.29	—	—	—	
Cocaine	7	8.4	±1.59	4	3.2	±0.88	0.02

ing one (P < 0.01). After denervation the threshold potentiating dose of serotonin was reduced whereas that of bufotenine remained unchanged. Nevertheless the potentiating threshold dose of serotonin was still greater than that of bufotenine. In denervated structures both contracting and potentiating doses of bufotenine were equal (P > 0.3); on the contrary, under analogous experimental conditions the serotonin potentiating dose was greater than the contracting one (P < 0.001).

In 3 experiments serotonin and bufotenine threshold doses for potentiation were alike whether norepinephrine or epinephrine was used as the stimulating drug.

In 4 experiments the potentiating effect of serotonin was compared to that of tryptamine. The effects of tryptamine were similar to those of serotonin but more fleeting.

BAS and BAB, 2 other tryptamine derivatives, not only did not potentiate the epinephrine contractions of the nictitating membrane, but 4 mg/kg of either drug inhibited the epinephrine effect. In a few experiments the antiserotonin activity of BAS and BAB was also investigated but no clearcut antagonistic effect toward serotonin could be seen in our records.

Psychotomimetic drugs. Mescaline, harmine and LSD-25 had a definite potentiating effect on epinephrine response (table 2), in increasing order of effectiveness. In table 2 the potentiating threshold doses of a typical adrenolytic drug, Dibenamine, and of a classical and very efficient potentiating drug, cocaine, are also reported. Both drugs displayed a potentiating effect similar to that of LSD-25. The threshold dose of cocaine is significantly greater than that of LSD-25 (P < 0.01) and Dibenamine (P < 0.02).

The potentiating dose of cocaine, in contrast to that of LSD-25 (figure 4), was lowered by denervation of the nictitating membrane (table 2).

LSD-25 analogues: In order to obtain information about the correlation between epinephrine sensitizing capacity and psychotomimetic efficiency, we studied a series of lysergic acid derivatives comprising sympatholytics (DHE and ergotamine), psychotomimetics (LSD-25 (Stoll, 1947), LSM (Gogerty and Dille, 1957), ALD 52 and LAE 32 (Rothlin, 1957b), Mether-

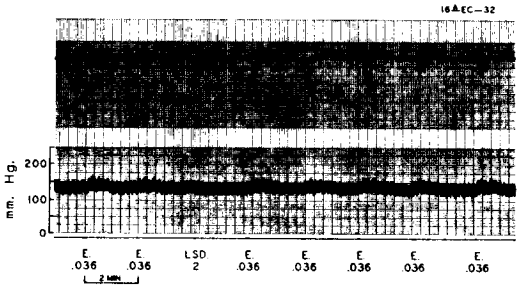


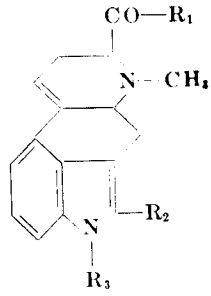
FIG. 4. The response of the denervated nictitating membrane to injected epinephrine is potentiated by a threshold dose of LSD.

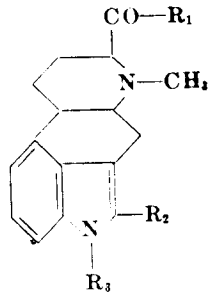
No changes in epinephrine circulatory effects; cat (3 kg) anesthetized as in fig. 1. Top: nictitating membrane; bottom: blood pressure. E = epinephrine; LSD = LSD-25; figures = μg/kg, i.v.

TABLE 3

Threshold doses (μg/kg) of LSD analogues potentiating epinephrine response

		R_1	R_2	R_3	Mean (μg/kg)	S.E.	No. of Animals
LSD	A	$-N(CH_2-CH_3)(CH_2-CH_3)$	H	H	1.42	±0.57	6
LSM	A	$-N(CH_2-CH_2-O-CH_2-CH_2)$	H	H	1.79	±0.21	4
ALD-52	A	$-N(CH_2-CH_3)(CH_2-CH_3)$	H	C_2H_5	2.40	±0.32	4
LAE-32	A	$-N(CH_2-CH_3)H$	H	H	1.86	±0.31	4
BOL	A	$-N(CH_2-CH_3)(CH_2-CH_3)$	Br	H	149.00	±32.76	5
Ergotamine	A	Peptide	H	H	2.10	±0.33	5
DHE	B	Peptide	H	H	1.30	±0.87	4
Methylergobasine	A	$-N(CH_3)-CH(H)-CH_2-CH_2OH$	H	H	1.60	±0.15	4





gine[4]) and an almost inactive drug, BOL-148. Table 3 shows the chemical structures and potentiating threshold doses of these drugs. Their doses did not differ significantly except for BOL, which was less active ($P < .001$).

However, with larger doses of the lysergic acid derivatives some differences in effects were seen. As reported in table 4, the response of the nictitating membrane to epinephrine was in-

[4] The injection of large doses of Methergine, 2 to 2.5 mg/kg i.v., causes abnormal behavior in cats (Brown and Dale, 1935).

hibited with 72.5 and 78 μg/kg, i.v., of DHE and ergotamine, respectively, while both Methergine, 125 μg/kg i.v., and LSD, 100 μg/kg i.v., still displayed potentiation of epinephrine. The potentiation obtained with 125 μg/kg of Methergine was prolonged as was that after bufotenine, LSD-25 and cocaine. Both Methergine, 125 μg/kg, and LSD-25, 100 μg/kg, potentiated the epinephrine effects on blood pressure. Finally, premedication with ergotamine (78 μg/kg, 30 min before) regularly prevented the epinephrine hypersensitivity of nictitating membrane induced by LSD-25. This phenomenon has not yet been thoroughly investigated.

TABLE 4

Modification of the epinephrine contraction of nictitating membrane in the cat by premedication with lysergic acid derivatives

No. of Animals*	Drug Tested	Dose	No. of Epinephrine Responses Showing 50% Inhibition	No. of Epinephrine Responses Showing a Potentiation
		μg/kg		
4	Ergotamine	78	17	—
4	DHE	72.5	26	—
4	Methylergonovine	125	—	40
4	LSD-25	100	—	40

* Each animal was injected 10 times with a threshold dose of epinephrine over a period of 20 minutes following the injection of the lysergic acid derivative. The table shows how many of the 40 responses of each group of 4 animals were inhibited or augmented.

Discussion. Hypersensitivity of the nictitating membrane to epinephrine can be produced by a large number of drugs: among others, methylatropine (Cervoni *et al.*, 1956), antihistaminics of different structures (Lecomte and Fischer, 1952), methylene blue (West, 1951) and adrenochrome (Lecomte and Fischer, 1951).

The following criteria may help to differentiate the epinephrine potentiation caused by psychotomimetics from various types of pharmacologically induced hypersensitivities toward epinephrine. 1) The presence of sympathetic tonus or that of a physiological transmitter is not necessary for the development of epinephrine sensitization. Thus the absence of potentiation by methylatropine (Cervoni *et al.*, 1956) in denervated structures may be contrasted to the effectiveness of cocaine, LSD-25, bufotenine and serotonin in similar experimental conditions (tables 1, 2; figure 4). 2) Very small doses are sufficient to elicit potentiation. This fact distinguishes the psychotomimetics we studied from antihistaminics, adrenochrome and methylene blue which have potentiating threshold doses several hundred times greater than that of bufotenine, harmine, lysergic acid derivatives, cocaine and Dibenamine (tables 1, 2, and 3). 3) The sensitizing effect remains qualitatively unaltered regardless of the dose used (table 4). An irreversible and long-lasting hypersensitivity to injected epinephrine in our experiments was displayed by such effective psychotomimetics as LSD-25, bufotenine, harmine and mescaline. BAS (Ruby *et al.*, 1958), BAB and BOL-148, which do not cause psychotomimetic effects, were inefficient or did not increase at all the epinephrine response. DHE and ergotamine, which induced hypersensitivity or antagonism according to the dose injected (tables 3, 4), fail to cause abnormalities in mentation.

We did not study the effect of high doses of Dibenamine but it is known that the blockade of the epinephrine stimulation of nictitating membrane develops more slowly and less effectively than that of the pressor response (Nickerson, 1949). Yet in man Dibenamine can act as a psychotomimetic agent (Sollmann, 1957).

A strict correlation between the epinephrine potentiation, defined according to the above mentioned criteria, and psychotomimetic effects does not exist, as is shown by Methergine. Nevertheless, it seems clear that there is a relationship between psychotomimetic effects and epinephrine sensitization at a peripheral site of action. This suggests the hypothesis that some similar action is responsible for the central effects of the psychotomimetics, *i.e.*, epinephrine potentiation might be one of the causes of disequilibrium in the activities of neurohormonal transmitters in the brain. This hypothesis is obviously but one of many. Much work remains to be done in clarifying these relationships.

No particular chemical group can be considered essential for the property of inducing hypersensitivity to epinephrine. Probably it could be pointed out that for structures containing an indole ring the presence of a chemical group attached to the carbon in the 2 position of the indole is not favorable for epinephrine sensitiza-

tion. BAB and BAS (2 methyl indoles) are inactive and BOL-148 (2-brom-LSD) is several times less active than any of the other indole derivatives tested. The fact that sympatholytics even in very low doses potentiate the epinephrine effect on nictitating membrane recalls similar observations by Cannon and Bacq (1931), Bacq and Fredericq (1935), Herwick *et al.* (1939), as well as by Jang (1941), who suggested that "the capacity to potentiate or to antagonize adrenaline can no longer be considered specific. . . ." This basic problem certainly deserves further examination.

SUMMARY

A quantitative analysis of the epinephrine potentiating effect on the nictitating membrane in the cat by psychotomimetic and chemically related drugs was performed. The potentiating threshold doses (μg/kg i.v.) of the drugs tested were: serotonin, 114.0; bufotenine, 5.5; mescaline, 152.0; harmine, 58.0; Dibenamine, 2.0; cocaine, 8.4; LSD-25, 1.42; LSM, 1.79; ALD-52, 2.4; LAE-32, 1.36; BOL-148, 149.0; ergotamine, 2.1; DHE, 1.3; methylergonovine, 1.6. Denervation of the nictitating membrane modified significantly the threshold doses of serotonin and cocaine. The potentiating effect of bufotenine, harmine, mescaline, cocaine, LSD and Methergine endures longer when doses greater than the threshold ones are injected. The potentiation by ergotamine and DHE can be seen only within a limited range of doses: after 70 μg/kg i.v., an antagonistic effect takes place.

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