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POTENTIATION OF HISTAMINE AND INHIBITION OF DIAMINE OXIDASE BY CATATONIC DRUGS

E. A. CARLINI, M. R. P. SAMPAIO, MEIRI SANTOS and GLACI R. S. CARLINI

Instituto Biológico, São Paulo, Brazil

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Abstract—It has been demonstrated that mescaline enhances histamine responses on rat blood pressure and on the guinea pig ileum. On the other hand, papaverine and bulbocapnine were found to potentiate the effects of histamine on the permeability of the capillary vessels of guinea pigs; moreover, papaverine when injected with histamine or histidine develops a catatonic state in mice. At concentrations of 1×10^{-5} and 1×10^{-4} M, papaverine and mescaline inhibit the histaminolytic power of diamine oxidase.

It is suggested that these effects of papaverine, mescaline, and bulbocapnine are mediated through an inhibition of histaminolysis, which in turn depends on the 4-methoxyphenylethylamine residue of these drugs.

IT HAS been reported that several drugs with a 4-methoxyphenylethylamine residue are able to provoke a catatonic-like state in laboratory animals.[1, 2] Bulbocapnine, one of these drugs, is able to enhance histamine responses in several biological preparations[3] and to inhibit diamine oxidase (DAO) *in vitro*.[4] It has also been reported that the catatonia caused by bulbocapnine is antagonized by the antihistamine, diphenhydramine.[5] On the other hand, it is known that catatonia is often associated with human schizophrenia, and several investigators have pointed out a relationship between schizophrenic state and a relative insensitivity to histamine[6–11] or a high blood level of this amine.[12, 13] Recently, 3,4-dimethoxyphenylethylamine, a compound closely related to the residue mentioned above, has been isolated from the urine of schizophrenic patients.[14, 15] Histamine is found and synthetized mainly in the hypothalamus and median eminence[16, 17] and has a typical subcellular distribution in brain.[18, 19]

These observations suggest that histamine may play a role in the function of the central nervous system, and that catatonic drugs possessing the 4-methoxyphenylethylamine residue could be acting, at least in part, through an alteration of this histaminergic mechanism. The present investigation was undertaken in an attempt to verify this hypothesis. Mescaline, papaverine, and bulbocapnine were tested for their ability to potentiate histamine responses on a few biological preparations. Also, the activity of these drugs in inhibiting the histaminolytic power of DAO is reported in this paper.

METHODS

Hog kidney diamine oxidase, histamine diphosphate, and mescaline sulfate (Nutritional Biochemicals Corp.); papaverine hydrochloride (Merck Laboratories); and bulbocapnine hydrochloride (Mann Research Laboratories) were used in the experiments. The amounts are expressed in terms of the bases.

The effects of mescaline, papaverine, and bulbocapnine on the histamine responses were measured on the rat blood pressure, on the guinea pig ileum, on the capillary permeability of guinea pigs, and on the spontaneous activity of mice. The catatonic state demonstrated by mice under the action of these drugs also were measured.

Rats were anesthetized with 1·5 g ethyluretane/kg i.p., and the carotidean blood pressure was recorded on a kymograph with a Condon mercury manometer.[20] Drugs were injected through a polyethylene cannula inserted into one external iliac vein.

Guinea pig ilea were suspended in a 10-ml chamber containing aerated Tyrode's solution at 37°. Contractions were recorded on a smoked drum with a frontal lever giving 4-fold magnification. To measure capillary permeability, adult guinea pigs were intravenously injected with 0·5 ml of 0·5% solution of trypan blue, and 5 min later the intradermal injections of the drugs under study were performed in 0·1 ml of 0·9% saline. One hour after the last injection the animals were decapitated and the skins removed. The spots produced by the dye in the under surface of the skins were measured in their two largest diameters and taken as indication of the capillary permeability.

To measure spontaneous activity mice were injected i.p. with the drugs under study and left alone in a swinging cage which registered the motions of the animals on a smoked drum. Catatonia was measured by lifting the forepaws of mice onto a horizontal rod 4 cm from the floor of the cage; three times every 10 min up to 100 min after the administration of the drugs, the mice were placed in this position. The total time, within each 10-min period, that the mice held the position was measured with a stopwatch.

The histaminolytic activity of DAO was measured either by a biological method or by the microvolumetric method of Kapeller-Adler.[21] In the biological method, 1 mg of the enzyme was incubated with 3 μg histamine at 37°; the volume of the mixture was completed to 10·0 ml with Sorensen's phosphate buffer, pH 7·0. Aliquots were taken 30, 60, 120, 180, and 240 min later and tested on a guinea pig ileum for residual histamine. A control tube with histamine and phosphate buffer was carried through in all determinations.

RESULTS

Table 1 and Fig. 1 illustrate the activity of mescaline on the hypotensive response of histamine in the rat. At the dosages employed mescaline produced a temporary increase in blood pressure (Fig. 1, B), and in order to avoid a possible interference of this pressor change in the analysis of the potentiation, the figures in Table 1 were taken when the blood pressure had returned to levels near to those observed before the injection of mescaline. An average increase of 68 and 104% in the histamine hypotension was obtained by injecting respectively 12 and 24 mg mescaline/kg in five nonpregnant rats; however, the alkaloid failed to promote any potentiation in two pregnant rats even after the injection of 80 mg/kg. Increasing the amount of mescaline up to 48 mg/kg in the nonpregnant rats did not enhance the potentiation.

TABLE 1. POTENTIATION OF HISTAMINE RESPONSE IN RAT BLOOD PRESSURE BY MESCALINE

Mescaline (mg/kg i.v.)	No. of experiments	Rats showing potentiation	Per cent of potentiation	Remarks
12	5	4	68 ± 34	Potentiation doubtful in 5th rat
24	5	4	104 ± 61	*Idem.*
48	2	2	16 and 66	
80	2	0	0	Pregnant rats

The reactivity of the guinea pig ileum to histamine, after the addition of mescaline, was also enhanced although not in all experiments. When present, the potentiation was achieved with 10 to 20 μg mescaline added to the 10-ml chamber. Bulbocapnine, 20 μg added to the chamber, also enhanced the histamine response. Papaverine, a smooth-muscle relaxant was not tested on the guinea pig ileum. The actions of papaverine, mescaline, and bulbocapnine on the permeability of the capillary bed to histamine are summarized in Table 2. It is seen that papaverine and bulbocapnine

TABLE 2. CAPILLARY PERMEABILITY OF GUINEA PIGS TO HISTAMINE, AS INFLUENCED BY PAPAVERINE, MESCALINE, AND BULBOCAPNINE

Drug	Drug alone (cm^2)	Effect* when injected with 0·1 μg histamine (cm^2)	(cm^2)
None	0·1 ± 0·03	0·94 ± 0·71	0·92 ± 1·1
Papaverine			
1 μg	0·44 ± 0·60	3·41 ± 1·59	
5 μg	0·50 ± 0·83		3·33 ± 1·05
Mescaline			
1 μg	0·47 ± 0·57	1·20 ± 0·81	
5 μg	0·05 ± 0·10		1·43 ± 0·93
Bulbocapnine			
5 μg	0·18 ± 0·05		2·65 ± 1·51

* The figures represent the product of the two largest diameters of the spots ± S.D. All injections were made in 0·1 ml of 0·9% saline.

are able to increase 2·5- to 4-fold this activity of histamine; Fig. 2 illustrates one typical experiment with papaverine. Mescaline did not have a clear effect; in some preparations an enhanced permeability was obtained, in others no difference was found, and in a few the alkaloid partially inhibited the action of histamine.

Histamine, up to 100 mg/kg body weight, had no effect on the spontaneous activity or in developing catatonia in mice. Papaverine also, when injected in doses up to 15 mg/kg, had no effect; however, 25 mg and up of papaverine/kg caused both a catatonic state and a decrease in spontaneous activity. On the other hand, when papaverine and histamine were injected together, even in doses of 12·5 mg of each substance/kg, a striking catatonic state was observed; in these cases the spontaneous activity of the mice was also very diminished. Figure 3 shows the duration of catatonia of mice injected either with papaverine alone or with papaverine plus histamine.

Some preliminary experiments showed that histidine also potentiates the catatonia induced by papaverine. Figure 4 illustrates the effects of histamine, papaverine, and histamine plus papaverine on the spontaneous activity of mice.

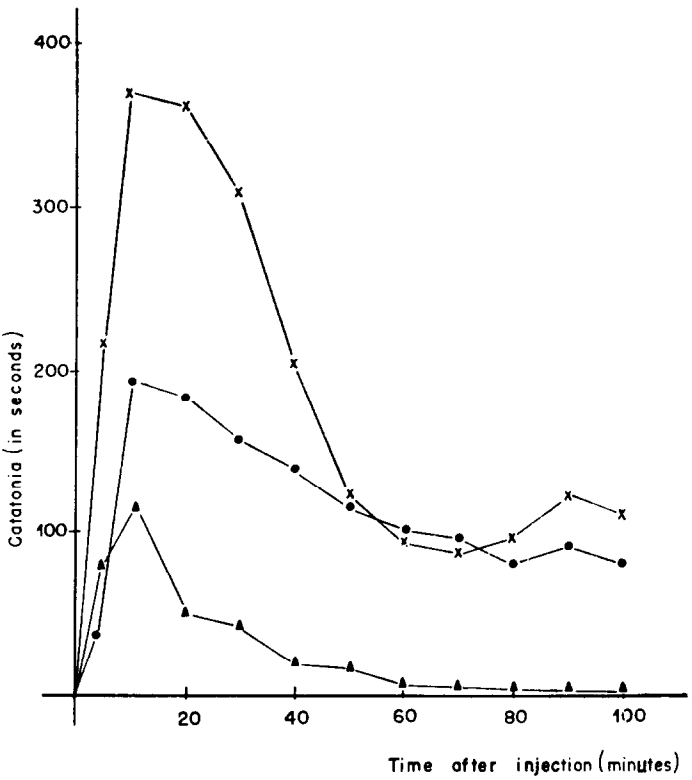

FIG. 3. Catatonia in mice after i.p. injections of 25 mg papaverine kg body weight (▲—▲), 12·5 mg papaverine + 12·5 mg/kg histamine kg (●—●), and 25 mg papaverine + 25 mg histamine /kg (×—×). The curves with 50 and 100 mg histamine/kg and 12·5 mg papaverine/kg are not shown because at these dosages no catatonia was observed. The data of each curve were obtained from at least ten animals.

Table 3 shows the percentage of histamine recovered, as measured by the biological method, after incubation with DAO, and demonstrates the influence of mescaline and bulbocapnine on the histaminolytic activity of the enzyme. It is seen that 1×10^{-4} M mescaline protected about 80% of histamine from destruction after 3-hr incubation. The enzymic inhibition was enhanced if mescaline was preincubated for 3 hr with DAO; under this condition the protection was almost complete after 4-hr incubation, even when 1×10^{-5} M mescaline was used. Figure 5 shows the activity of mescaline and papaverine on the histaminolytic power of DAO, as measured by the microvolumetric method of Kapeller-Adler.[21] Both alkaloids revealed inhibitory action on the enzyme, papaverine being about ten times more active.

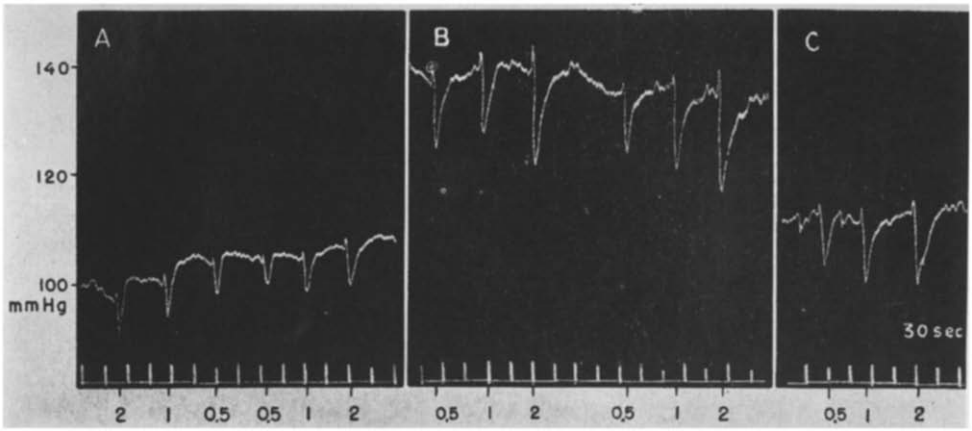

FIG. 1. Response of the rat carotidean blood pressure to histamine before (A) and 8 and 20 min (B and C) after the i.v. injection of 24 mg mescaline/kg body weight. Bottom numbers refer to micrograms of histamine injected through the iliac external vein (250-g male rat anesthetized with ethyluretane).

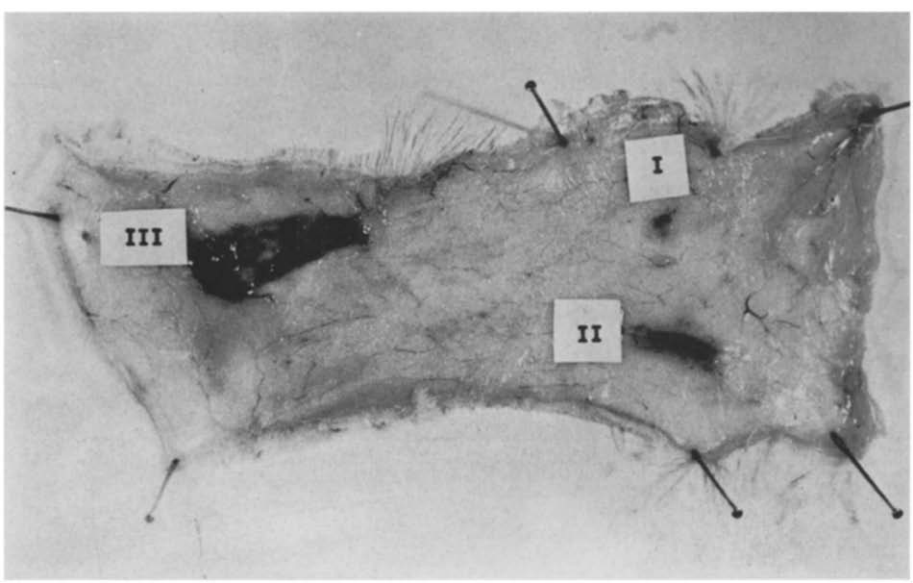

FIG. 2. Influence of papaverine on the capillary permeability of guinea pig to histamine. I, Intradermal injection of 5 μg papaverine. II, Intradermal injection of 1 μg histamine. III, Intradermal injection of 5 μg papaverine plus 1 μg histamine. All injections were made in 0·1 ml of 0·9% saline.

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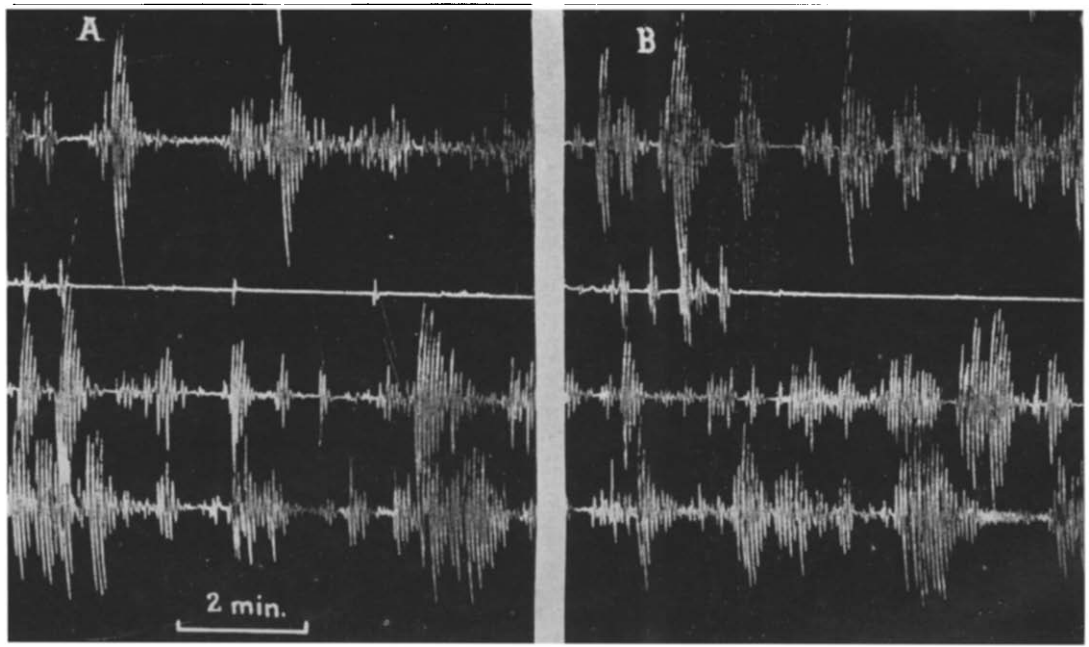

FIG. 4. Effect of histamine, papaverine, and histamine + papaverine on the spontaneous activity of four mice. From top to bottom: control mouse i.p. injected with 0·2 ml of 0·9% saline; mouse injected with 25 mg papaverine/kg + 25 mg histamine/kg; mouse injected with 25 mg papaverine/kg; mouse injected with 100 mg histamine/kg. A: spontaneous activity registered between 20 and 28 min after the injections. B: *Idem.*, 40 and 48 min.

TABLE 3. PERCENTAGE OF HISTAMINE RECOVERED AFTER VARIOUS INCUBATION PERIODS WITH DAO; INFLUENCE OF MESCALINE AND BULBOCAPNINE ON THE ENZYME ACTIVITY

Drug	No. of experiments	Preincubation enzyme-inhibitor (min)	Per cent of histamine recovered				
			Incubation time (min)				
			30	60	120	180	240
None	10		86·0 ± 9·6	76·2 ± 20·2	29·1 ± 23·1	22·1 ± 20·0	4·0 ± 4·2
Mescaline							
1×10^{-6}M	3	0	57·2 ± 13·1	47·3 ± 15·7	13·4 ± 7·2	7·2 ± 4.0	
1×10^{-6}M	3	180	98·0 ± 12·7	90·2 ± 18·2	40·8 ± 19·7	29·3 ± 13·4	
5×10^{-5}M	3	180	97·1 ± 10·3	100·2 ± 7·9	53·2 ± 8·9	32·7 ± 15·1	27·1 ± 14·6
1×10^{-5}M	2	0	101·1 ± 12·4	93·3 ± 18·1	62·1 ± 19·4	34·0 ± 18·5	
1×10^{-5}M	4	180	95·4 ± 23·7	102·7 ± 19·4	110·2 ± 21·9	95·0 ± 14·6	93·1 ± 13.4
1×10^{-4}M	4	0	92·9 ± 18·9	81·1 ± 13·4	83·4 ± 17·6	80·1 ± 26·9	
1×10^{-3}M	5	0	125·2 ± 21·4	126·8 ± 18·3	97·4 ± 15·1	109·2 ± 13·1	
Bulbocapnine							
1×10^{-6}M	1	0	69·1	53·4	8·0		
1×10^{-4}M	1	0	106·7	101·1	107·5	100·2	
1×10^{-3}M	1	0	123·1	131·0	134·0	130·2	

The residual histamine was measured on the guinea pig ileum.

DISCUSSION

We have found that mescaline potentiates histamine responses on rat blood pressure (Fig. 1, Table 1), and on the guinea pig ileum. Papaverine, on the other hand, was active in enhancing histamine activity on the capillary vessels of guinea pigs (Fig. 2,

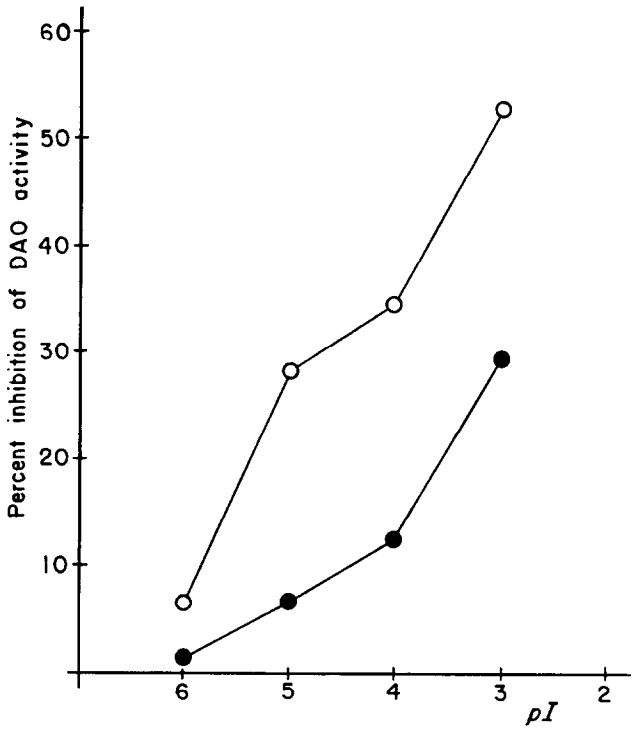

FIG. 5. Inhibition of DAO by papaverine (○—○) and mescaline (●—●). Preincubation was not performed between the enzyme and the inhibitors. Each point in both curves represents the mean of four experiments; pI: negative logarithm of the molecular concentration of the inhibitor.

Table 2). Furthermore, papaverine when given together with histamine or histidine caused a catatonic state and decreased the spontaneous activity of mice (Figs. 3 and 4); both drugs injected separately showed no marked effect. Bulbocapnine, the third substance studied, was also able to potentiate histamine responses; our data on bulbocapnine are in close agreement with those of others.[3]

One possible explanation for these facts emerges from the work of Lindell and Westling,[22] who showed that histamine potentiation can occur *in vivo* as well as *in vitro* by an inhibition of DAO. The inhibition described here of DAO by papaverine and mescaline at concentrations of 10^{-5} and 10^{-4} M supports the idea that potentiation is really due to this mechanism. Further indication that DAO inhibition may play a role comes from the fact that in two pregnant rats potentiation by mescaline was not obtained; since, during pregnancy, the levels of DAO can reach values much above the normal,[23, 24] the enzyme inhibition in this condition must be more difficult to attain. In fact, we failed to potentiate histamine responses in pregnant animals even by using mescaline up to 80 mg/kg.

Mescaline, papaverine, and bulbocapnine, which are known to bring about a

catatonic state in laboratory animals,[1, 2] have a common residue which is closely related to 3,4-dimethoxyphenylethylamine isolated from the urine of schizophrenic patients.[14, 15] Furthermore, it has been reported that mescaline and bulbocapnine are synergistic in producing hypokinesis in rats,[25] and that 3,4-dimethoxyphenylethylamine produces a clear catatonic state, sometimes preceded by tremors, when injected subcutaneously into cats.[2] The data point to the possibility that, at least in part, the catatonic activity of these drugs is being mediated through a common mechanism such as inhibition of diamine oxidase. Recently, Kapeller-Adler and MacFarlane[26] successfully separated the enzyme histaminase from a hog kidney DAO preparation. They have further proved that the electrophoretically pure histaminase was free from contamination with DAO, inactive on diamines, and specifically active on histamine and its N-methyl-substituted derivatives. DAO, on the other hand, proved to be inactive on histamine. Therefore, in view of these findings, the possibility is now being investigated that the enzymic inhibition described in this and other papers[4, 27] is actually an action on histaminase which contaminates hog kidney DAO preparations.

The fact that histamine under special conditions is able to induce catatonia,[28] the knowledge that bulbocapnine-catatonia can be neutralized by diphenhydramine,[5] and the data presented here suggest that the mechanism of action of catatonic drugs with a 4-methoxyphenylethylamine residue might occur through a rise of histamine or its N-methyl-derivative concentrations in brain, resulting from an inhibition of histaminolysis.

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