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# INHIBITION OF DIAMINE OXIDASE BY BULBOCAPNINE*

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**Abstract**—Using a modification of the spectrophotofluorometric method of Shore, Burkhalter and Cohn, an inhibition of diamine oxidase by bulbocapnine, using histamine as a substrate, has been demonstrated. The mechanism of the inhibition is competitive, as indicated by Lineweaver–Burke and Hunter–Downs plots. Pyridoxal phosphate was capable of antagonizing the inhibition by bulbocapnine of dialyzed hog kidney diamine oxidase. The possible involvement of a histaminergic mechanism in experimental catatonia is discussed.

BULBOCAPNINE is an alkaloid obtained from the plant *Corydalis cava*. It is of special interest because, upon administration to lower animals and man, it produces a state of altered behavior variously described as catatonia or plastic rigidity. De Jong[1] made a very extensive study of the behavorial effects of this drug. He called attention to the close physical resemblance between bulbocapnine-induced catatonia and the idiopathic variety sometimes associated with human schizophrenia; Busciano[2] also has commented on this similarity. Observations by a number of laboratory and clinical investigators have indicated that there may be a relationship between the schizophrenic state and an altered state of reactivity to histamine.[3–11]

Our present interest in bulbocapnine and its influence on diamine oxidase was initated by the observation in our laboratory‡ that bulbocapnine enhances the response of the isolated guinea pig ileum to histamine. In support of this observation, a potentiating effect by bulbocapnine on the vasoconstrictor activity of histamine on the isolated perfused rabbit ear was demonstrated.[12]

We were led to investigate the effect of bulbocapnine on diamine oxidase by the work of Arunlakshana, Mongar and Schild,[13] who showed that one *in vitro*-mechanism (isolated guinea pig ileum) whereby the effect of histamine can be potentiated is by the inhibition of diamine oxidase. The work of Lindell and Westling[14] indicated that *in vivo*, as well as *in vitro*, histamine responses can be enhanced by the inhibition of diamine oxidase.

Our present investigation was designed to test the hypothesis that bulbocapnine is an inhibitor of diamine oxidase *in vitro*.

## METHODS

We designed experiments that used commercially obtained hog kidney diamine oxidase (1 mg; final concentration, 0·142 mg/ml, Nutritional Biochemical Corp:

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‡ This observation was made in our laboratory by Dr. Carroll M. Smith.

Lot number, 1142) buffered to pH 7·2 with Sorensen's phosphate buffer prepared by mixing the proper amounts of 0·067M $KH_2PO_4$ and 0·067M $Na_2HPO_4$. Bulbocapnine hydrochloride or isonicotinic acid hydrazide (base) was added and preincubated with the enzyme for 15 min prior to the addition of the histamine substrate (3 $\mu$g; final concentration of histamine base, 0·42 $\mu$g/ml). The experimental mixture was incubated in a Dubnoff Metabolic Shaker at a constant temperature of 37 °C. Suitable standards and blanks were carried through the entire procedure. Extraction and spectrophotofluorometric analysis (Aminco–Bowman Spectrophotofluorometer) for residual histamine were carried out by our modification of the method described by Shore *et al.*[15] This modification entails a preliminary extraction of the experimental mixture with 15 ml of chloroform after adjustment of the pH to 6·5; subsequently, the chloroform extracted aqueous phase was analyzed for histamine according to the method of Shore *et al.*

The necessity for the preliminary extraction of the experimental mixture into chloroform is indicated in Fig. 1, which illustrates the interfering effect of bulbocapnine on the spectrophotofluorometric assay for histamine; at pH 6·5 the bulbocapnine is almost completely extracted and residual bulbocapnine does not interfere with the histamine assay.

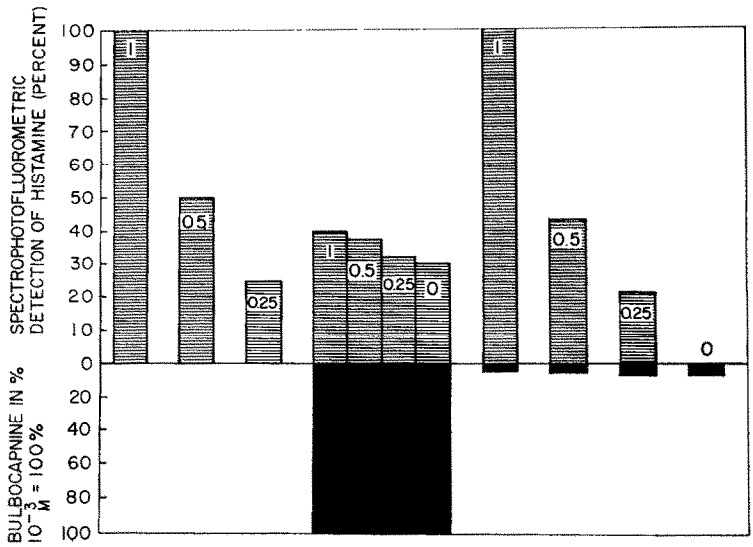


FIG. 1. The quenching effect of bulbocapnine on the spectrophotofluorometric assay of histamine. Values on the bars represent concentration of the original stock histamine in $\mu$g/ml. Recovery is calculated in terms of per cent, 100 per cent indicating the recovery of 1 $\mu$g of histamine, when diluted to a final concentration of 0·42 $\mu$g/ml. The solid bars represent the concentration of bulbocapnine determined by reading the fluorescence of the alkaloid at 480 m$\mu$ after activation at 300 m$\mu$.

The activity of our commercially obtained diamine oxidase is shown in Table 1 which indicates the activity of the enzyme with histamine as the substrate.

## RESULTS

The inhibitory activity of bulbocapnine on diamine oxidase, as compared with isonicotinic acid hydrazide, a known inhibitor of diamine oxidase,[16] is illustrated in

Fig. 2. A concentration of isonicotinic acid hydrazide of $5 \times 10^{-6}$ M inhibited the enzyme by 50 per cent, as did a concentration of bulbocapnine of $8 \times 10^{-5}$ M.

The effect of time and molar concentrations of bulbocapnine on the activity of diamine oxidase is illustrated in Fig. 3, in which the percent of residual histamine is

TABLE 1. THE HISTAMINOLYTIC ACTIVITY OF COMMERCIALLY OBTAINED HOG KIDNEY DIAMINE OXIDASE

| Preparation of diamine oxidase (mg) | Histamine ($\mu$g) | Histamine destroyed (%) |
|---|---|---|
| 3 | 3 | 100 |
| 2 | 3 | 95 |
| 1 | 3 | 67 |
| 0·5 | 3 | 44 |

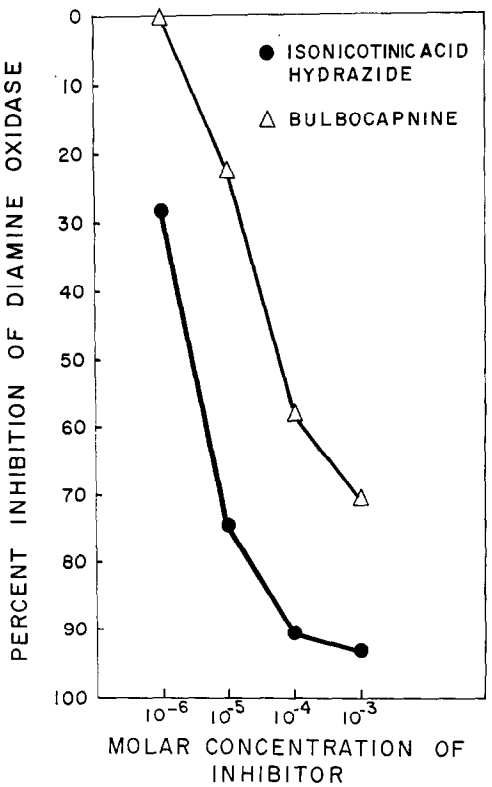


FIG. 2. The inhibitory capacity of bulbocapnine and isonicotinic acid hydrazide on diamine oxidase after incubation for 30 min with histamine.

plotted against time. It can be observed that bulbocapnine in concentrations of $10^{-3}$ M, $10^{-4}$ M and $10^{-5}$ M, respectively, was inhibitory, while $10^{-6}$ M was without such an effect. The mean percentage of histamine recovered, together with the standard error of the mean, as influenced by time and molar concentration of bulbocapnine, is shown in Table 2.

After incubation for 30 min, the level of significance between control and experimental residual concentrations of histamine, as defined by *P*-values, was 0·001 for $10^{-3}$ M and $10^{-4}$ M, and 0·05 for $10^{-5}$ M, for bulbocapnine. Concentrations of bulbocapnine of less than $10^{-5}$ M did not cause significant inhibition of diamine oxidase, and

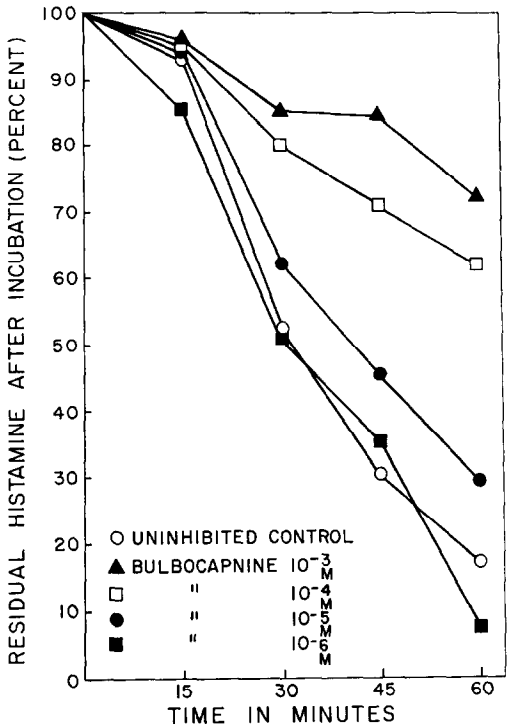


FIG. 3. The inhibitory capacity of bulbocapnine on diamine oxidase, as represented by the amount of residual histamine after incubation for periods of varying duration. Each point represents the mean of eleven experiments.

TABLE 2. RESIDUAL HISTAMINE AFTER INCUBATION WITH DIAMINE OXIDASE

| Bulbocapnine (M) | Incubation time | | | |
|---|---|---|---|---|
| | 15 min | 30 min | 45 min | 60 min |
| Control | 93·00 ± 1·2 | 52·25 ± 0·2 | 30·05 ± 4·8 | 17·37 ± 3·1 |
| $10^{-3}$ M | 96·00 ± 2·7 | 85·75 ± 1·7 | 85·75 ± 3·2 | 72·13 ± 3·1 |
| $10^{-4}$ M | 95·63 ± 1·6 | 80·00 ± 1·8 | 71·03 ± 2·3 | 62·38 ± 3·5 |
| $10^{-5}$ M | 94·06 ± 1·7 | 62·74 ± 2·2 | 45·15 ± 1·7 | 29·68 ± 1·5 |
| $10^{-6}$ M | 86·00 ± 4·0 | 51·56 ± 2·4 | 35·00 ± 2·4 | 8·75 ± 5·0 |

* Expressed in per cent of 1 μg of histamine base recovered (final concentration of histamine: 0·42 μg/ml (± standard error of the mean)). Each value represents the mean of eleven experiments.

none of these concentrations resulted in significant inhibition during an incubation period of 15 min.

Having determined that bulbocapnine is an inhibitor of diamine oxidase *in vitro* in this test system, a determination of the nature of this inhibition, i.e. whether competitive or non-competitive, was made. In exploring the mechanism of inhibition, two

methods were used: that of Lineweaver and Burk[17] and that of Hunter and Downs.[18] Using both methods, we obtained plots from five separate experiments which are indicative of a competitive type of inhibition. Fig. 4 is a representation of the method used by Hunter and Downs to describe inhibition; according to this method, a horizontal line describes non-competitive inhibition, whereas competitive inhibition is indicated by a diagonal line.

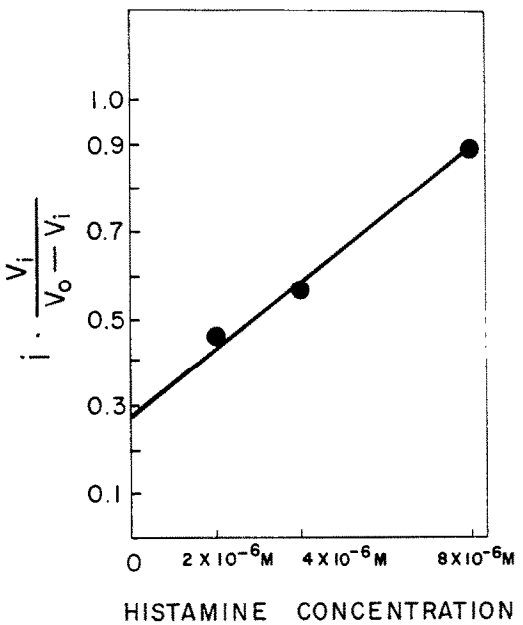


FIG. 4. Hunter–Downs plot demonstrating a competitive type of inhibition of diamine oxidase by bulbocapnine. I = $10^{-3}$ M bulbocapnine. Incubation time, 30 min.

Since pyridoxal phosphate is a cofactor of diamine oxidase,[19] and in view of the report of Davison[19] that the inhibitory capacity of isonicotinic acid hydrazide on diamine oxidase is less effective in the presence of an excess of pyridoxal phosphate, the effect of this cofactor on the inhibition of diamine oxidase by bulbocapnine was investigated. The commercial enzyme powder was dialyzed for 24 h against distilled de-ionized water and the inhibitory capacity of bulbocapnine was examined in the presence of 1, 2, 4, 8, and 16 $\mu$g, respectively, of pyridoxal phosphate per ml. In five separate experiments, the inhibition of diamine oxidase was less effective in the presence of pyridoxal phosphate. In the presence of 1 $\mu$g, pyridoxal phosphate, inhibition diamine oxidase by $10^{-3}$ M bulbocapnine 43·4 $\pm$ 4·4 (mean per cent $\pm$ S.E.) less effective, with 2 $\mu$g/ml pyridoxal phosphate the enzyme inhibition was 62·0 $\pm$ 2·8 (mean per cent $\pm$ S.E.) less effective. Higher concentrations of pyridoxyl phosphate were not more effective in reversing the inhibition of the enzyme.

## DISCUSSION

Inhibition of diamine oxidase *in vitro* by bulbocapnine in concentrations of $10^{-3}$ M, $10^{-4}$ M and $10^{-5}$ M, respectively, has been demonstrated. After incubation for 30 min, there are significant differences between control and experimental values,

as indicated by *P*-values of 0·001 for concentrations of the inhibitor of $10^{-3}$ M and $10^{-4}$ M.

The association in a single agent, bulbocapnine, of the capacity to produce an altered behaviorial state which resembles the catatonia often associated with human schizophrenia, together with the potential to alter histamine levels through the inhibition of diamine oxidase, permits speculation concerning this phenomenon and the potential histaminergic nature of this alkaloid. Both histamine[20] and diamine oxidase[21] are present in mammalian brain. The fact that the catatonia produced by bulbocapnine can be antagonized by the antihistaminic agent, diphenhydramine,[22, 23] adds further support to a hypothesis which involves a central histaminergic mechanism for the bulbocapnine-induced catatonia.

We feel that to assert that bulbocapnine is active in producing behavorial changes characterized by the catatonic state only through a histaminergic mechanism, mediated through diamine oxidase inhibition, would be premature at this time. There are other diamine oxidase inhibitors which are more potent than bulbocapnine in this activity and these inhibitors have not been reported to produce the catatonic state. However, we have shown that bulbocapnine is active in the inhibition of diamine oxidase *in vitro*. We have described other effects of bulbocapnine (epinephrine and serotonin blockade)[24] which by themselves, or combined with the diamine oxidase inhibitory capacity of bulbocapnine, may alter the function of the central nervous system. Studies relating these actions of bulbocapnine to the levels of biologically active amines in the central nervous system are now in progress.

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