THE RELEASE OF HISTAMINE BY SINOMENINE*

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Sinomenine is an alkaloid contained in the root of *Sinomenium acutum* Rehd. et Wilson (Fam. Menispermaceae), growing wild in southern Japan, and has long been used as a home remedy for neuralgia and rheumatism. It has a chemical structure similar to that of morphine, as shown left (1). Ishiwari (2, 3), who first isolated this alkaloid and studied its pharmacological actions, found that sinomenine caused depression of blood pressure by dilating peripheral blood vessels in mammals and acceleration of thoracic lymph flow in dogs. He (4) assumed that the foregoing therapeutic effect of this alkaloid was probably due to the increased circulation in the locus by such peripheral vascular effect.

Augmentation of lymph rich in protein caused by sinomenine (5) is also observed in the case of histamine (6), as well as with peptone (7, 8) and anaphylaxis (9) where histamine liberation is known to occur. Depression of arterial pressure by sinomenine is generally accompanied by the increase of portal pressure and liver volume (5), which phenomena are also known with peptone and in anaphylaxis (10). Recently, MacIntosh and Paton (11) observed that a marked liver congestion was caused by some organic bases which liberate histamine. The known side-effects of sinomenine are erythema, urticaria, pruritus, and facial swelling (4, 12).

In the previous report (13), the author found that the lymphagogic and liver congestive actions of this alkaloid, and the wheal caused by its intradermal injection are notably reduced by the pretreatment or combination with Benadryl.

The present series of experiments were undertaken in order to solve the problem of whether the afore-mentioned effect of sinomenine observed in dogs were the result of liberation of histamine.

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METHODS

A dog, withheld from food for the previous 24 hours, was anesthetized by subcutaneous injection of a morphine-urethane mixture. The arterial blood pressure was recorded by the usual method from femoral artery with 10 per cent sodium citrate solution as an anticoagulant. Blood samples were withdrawn generally from the femoral vein, into a syringe containing 0.05 cc of 1 per cent sodium heparin solution for each cc of blood, centrifuged without delay, and their plasma were used for pharmacological assays. Lymph was allowed to flow from the cannula inserted into the thoracic duct and the samples obtained successively by changing the receiving vessel every 10 minutes were offered for tests. Histamine assay was carried out on an isolated ileum of a guinea-pig suspended in a 10 cc bath containing the Tyrode solution and by the arterial blood pressure of a chloralose-anesthetized and atropinized cat. The samples were either used in a crude state or as an extract for histamine by Code's (14) modification of the method of Barsoum and Gaddum (15). The samples were used in a crude state or as an extract for histamine by Code's (14) modification of the method of Barsoum and Gaddum (15). The samples were used in a crude state or as an extract for histamine by Code's (14) modification of the method of Barsoum and Gaddum (15). The samples were used in a crude state or as an extract for histamine by Code's (14) modification of the method of Barsoum and Gaddum (15).

RESULTS

Vasodepressor effects. In general, systemic venous injection of 0.1 to 0.3 mg/kg dose of sinomenine gave a transitory and slight fall in arterial pressure, but in 1 to 2 mg/kg dose, a profound fall was witnessed, and in 3 to 5 mg/kg dose, the fall was of long duration or gave an irrecoverable shock. The degree of the depressor response was extremely varied by individuals; in some cases, 0.1 mg/kg dose has given a fall of over 50 mm Hg that lasted for more than 30 minutes, while 11 (14 per cent) out of 78 dogs gave completely refractory results to a 3 mg/kg dose. The pulse rate became slightly slower at the beginning of vasodepression and slightly rapid at the time of recovery, but heart beats were strong even at the time of profound fall in blood pressure. The volume of the hind limb was found to have increased oncometrically during the fall of blood pressure. The dog, in which a profound vasodepression had been caused by this drug, was completely immune to the second injection given after its recovery. However, when the fall in blood pressure was rather small, the repeated injection did cause some reaction, although much weaker than that by the first injection. Comparison of the vasodepressor effect by the alternate injection of such a small dose to the left and right auricles of a dog showed that the administration of the drug to the left auricle gave a stronger effect and that the period until the appearance of the depressor effect was a few seconds shorter. These results give rise to the assumption that the depressor
effect of this drug is due to the peripheral vascular action. Atropinization and severance of the vagi did not give any marked influence on the depressor effect. The notable fact obtained through these experiments was that there was a far longer latent period (20 to 50 seconds) until a precipitous fall began by sinomenine injection compared to the same caused by histamine (see Fig. 1). The two-phase fall reported by MacIntosh and Paton (11) in connection with certain histamine liberators was occasionally seen also by this drug. The fall of arterial blood pressure by sinomenine was not remarkably reduced by pretreatment with 10 mg/kg of Benadryl.

**Lymphagogic effect.** Lymph flow from the thoracic duct was found to be markedly accelerated for a period of 1 to 2 hours after the intravenous injection of 1 to 3 mg/kg of sinomenine. This had already been observed by Ishiwari (3), Kobayashi (5), and others. In this case, the portal pressure was elevated for a few minutes in less than 150 mm H_2O degree and the liver volume was found onometrically to increase definitely at the same time. These changes lasted during a far shorter periods than the fall of arterial pressure (see Fig. 2). The acceleration of thoracic lymph flow may be due to the increased plasma filtration in the liver. However, cervical lymph collected by McCarrell's (16) technique also showed a marked acceleration and, moreover, its protein concentration was higher. These suggested that the filtration of plasma through capillaries were heightened throughout the body. Such increase in portal pressure and liver volume, as well as the lymphagogic action were almost totally suppressed by the intravenous injection of 10 mg/kg of Benadryl 30 minutes prior to the administration of sinomenine. It seemed, however, that no such marked suppression of an increase in protein concentration of the lymph occurred by this pretreatment (see Fig. 3). Hematocrit was also markedly increased. Tachyphylaxis was evident in lymphagogic action, as in vasodepressor effect (cf. Fig. 2), and 3 (12.5 per cent) out of 24 dogs inherently showed no acceleration of lymph flow by 3 mg/kg dose of this drug. This ratio is approximately the same as the ratio of similar cases in vasodepressor response. This was further endorsed by the observation that in a few cases where blood pressure and lymph flow effects were examined at the

![FIG. 1. Dog, morphine-urethane. Arterial blood pressure. Hist. refers to an injection of 5 µg/kg of histamine hydrochloride by femoral vein; Sinom. refers to that of 0.3 mg/kg of sinomenine hydrochloride. Time marker = 5 sec. and 1 min. intervals](image-url)
same time, the strength of the two responses were completely parallel.

Cutaneous reaction. Intracutaneous administration of sinomenine in men and dogs caused wheal and flare responses that are hard to distinguish from those caused by histamine. Wheal can even be caused by a dilution of 1 in 500,000, and can be reduced or totally suppressed by the addition of Benadryl to this solution, but not as distinctly as the reduction of the wheal of same size caused by histamine. The area of the skin from which a wheal caused by sinomenine had disappeared remains immune to sinomenine for some time. In 1:100 dilution of sinomenine, the skin remained completely refractory for 24 hours, it necessitating 120 to 160 hours until the wheal of the same size could be caused. Such refractoriness cannot be observed with histamine and the latter caused a wheal of the same size as in normal skin on a skin refractory to sinomenine.
Comparison of the wheal dimension caused by various concentrations of sinomenine and histamine showed that 1 mg of sinomenine corresponded to approximately 0.46 mg of histamine in causing the wheal of the same dimension. If it is assumed that the histamine liberated in the skin possessed the same effect, weight for weight, as the histamine injected into the skin, then one molecule of sinomenine is seen to liberate approximately 1.5 molecules of histamine.

Identification of the depressor substance which appears in the lymph. Intravenous injection of a definite amount of thoracic lymph of a dog, notably augmented by the injection of sinomenine, in an atropinized and chloralose-anesthetized cat caused a precipitous fall of arterial pressure with a short latency, as in the case of histamine. Such action was seen only in a very slight degree in the lymph before the injection of sinomenine and was not caused if the cat had been given previous intravenous injection of 0.5 mg/kg of Neo-Antergan, similar to the depressor effect of histamine of a comparable strength. The effect of contracting the isolated ileum of a guinea-pig by the lymph was increased over an hour after the injection of sinomenine, the maximal effect being seen 20 to 30 minutes after the injection (Fig. 4a). This action was not suppressed to a great extent by atropine, as was the case with histamine of a comparable effect, but was inhibited by the pretreatment of the gut with an excess of histamine (15) or in the presence of Neo-Antergan, similar to the action of histamine of a comparable strength. However, the action of acetylcholine, Ba\(^{++}\) and K\(^+\) was practically unaffected by these pretreatments (Fig. 4b and c). Sinomenine itself at concentrations above 50 \(\mu g/cc\) showed a slight inhibitory effect on the ileum contraction caused by histamine and acetylcholine, but gave almost no effect on the tone by itself, even in \(10^{-3}\) dilution. It follows, therefore, that such activity of the lymph may be indicated by the equivalent concentration of histamine. Such a value obtained coincided with that obtained by the parallel assay by the blood pressure of cat, within an experimental error of the two methods. (As was reported by Code (14), the value obtained by the cat's blood pressure was generally higher than that by the ileum method.)

Finally, a lymph extract was prepared by the method of Code (14), involving the destruction through acid hydrolysis of most of the substances acting like histamine against guinea-pig's ileum and cat's blood pressure which are likely to be present in the tissue extract. Assay results obtained from this extract with two objects against histamine also coincided within the error of these methods. Moreover, coincidental value was also found, within a loss by extraction, with histamine equivalent obtained with a fresh lymph.

When the degree of lymph acceleration became large by the intravenous injection of sinomenine the increase of such active principle appearing in the
lymph also became extreme. On the contrary, when there was an inherent insensibility or in the case of tachyphylaxis, increase of an equivalent concentration of histamine could not be detected in the lymph by any of the assay methods.

\[ \text{FIG. 4. Guinea-pig's ileum in 10 cc bath. (a) Contractions due to 1 cc thoracic duct lymph before (cont.) and after an injection of 3 mg/kg of sinomenine hydrochloride by femoral vein. Figures designate sequence of successive 5 min. specimens after the injection. H=0.03 µg histamine. (b) and (c) Analysis of the effect of thoracic duct lymph after sinomenine. L=1 cc lymph, H=0.6 µg histamine, ACh=30 µg acetylcholine chloride, Ba=1 mg barium chloride, Atr=1 µg atropine sulfate in 10 cc bath. At arrow 3 µg histamine for 30 min.} \]

\textit{Vasodepressor substance appearing in the blood plasma.} The plasma from a dog showing notable vasodepressor response by sinomenine contained an increased amount of a substance that causes depression of blood pressure in a cat and contraction of a guinea-pig's ileum. This substance showed the same behavior as the principle of a similar action that appeared in the lymph against pretreatment with atropine and Neo-Antergan and resistance to acid inactivation. Such a substance was found in 1 to 3 µg/cc histamine equivalent directly after injection of 3 mg/kg of sinomenine when a fairly great depression of blood pressure was observed but about one-half of this effect was recovered after 30 minutes in spite of the fact that the hypotension persisted. This recovery steadily progressed when a second injection of sinomenine was given while the low blood pressure persisted (see Fig. 5 and Table 1). Such a fact makes it doubtful, for the
FIG. 5. Assay of histamine equivalents in blood plasma before and after an injection of 3 mg/kg of sinomenine hydrochloride: plasma from the experiment on the dog No. 245 in Table 1. Upper tracings: (a) guinea-pig's ileum, (b) cat's blood pressure: P₁, P₂, and P₃ are plasma withdrawn before and 1 min. and 30 min. respectively after the 1st injection, P₄ is plasma 1 min. after the 2nd injection of the same dose of sinomenine. H=histamine (base), N.A.=0.5 µg Neo-Antergan maleate. Lower tracings: (c) guinea-pig's ileum, (d) and (e) cat's blood pressure: P₁X, P₂X, P₃X, and P₄X refer to the extracts by Code's method corresponding to P₁, P₂, P₃, and P₄ respectively. cc and µg in (a) and (c) are quantities in 10 cc bath.

TABLE I. Histamine equivalents (µg/cc) of blood plasma from dogs treated with sinomenine hydrochloride (3 mg/kg)

<table>
<thead>
<tr>
<th>Dog</th>
<th>Specimen</th>
<th>Assay method</th>
<th>Before sinom.</th>
<th>1 min. after sinom.</th>
<th>30 min. after sinom.</th>
<th>1 min. after 2nd sinom. inj. made with 2 hrs. interval</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. 245</td>
<td>Fresh plasma</td>
<td>Cat's B.P. Guinea-pig's gut</td>
<td>0.28</td>
<td>1.30</td>
<td>0.77</td>
<td>0.39</td>
</tr>
<tr>
<td></td>
<td>Plasma extract</td>
<td>Cat's B.P. Guinea-pig's gut</td>
<td>0.25</td>
<td>1.23</td>
<td>0.74</td>
<td>0.37</td>
</tr>
<tr>
<td></td>
<td>Blood pressure (mm Hg)</td>
<td></td>
<td>114</td>
<td>64</td>
<td>62</td>
<td>66</td>
</tr>
<tr>
<td>No. 246</td>
<td>Fresh plasma</td>
<td>Cat's B.P. Guinea-pig's gut</td>
<td>0.14</td>
<td>3.20</td>
<td>1.32</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Plasma extract</td>
<td>Cat's B.P. Guinea-pig's gut</td>
<td>0.08</td>
<td>3.03</td>
<td>1.32</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Blood pressure (mm Hg)</td>
<td></td>
<td>123</td>
<td>54</td>
<td>40</td>
<td></td>
</tr>
</tbody>
</table>
time being, to conclude that the histamine equivalent that appeared in the blood plasma by this drug is the prime mover of vasodepressor effect caused by sinomenine.

As an attempt on solving this problem, intravenous injection of 1 to 2 mg/kg of histamine hydrochloride, a dose that can cause about a similar vasodepressor effect as in the foregoing experiments, was given to a dog and the amount of histamine in plasma was determined. The results are shown in Table 2. In the case, the plasma histamine immediately after the injection was similarly high, but the amount of histamine 30 minutes later showed a fairly rapid recovery in contrast to a very slight recovery of the blood pressure. This result suggests the possibility that the increase of histamine, liberated by sinomenine, in plasma can approximately indicate the degree of blood pressure depression in such a case. However, no adequate explanation can yet be given for the notably persistent low blood pressure even after the rapid disappearance of histamine from circulating blood. Setting aside the question of whether or not these chemicals give some injurious effect to the wall of the blood vessel, the above phenomenon may be caused by the maintenance of a certain concentration of the substance that causes dilation of blood vessels for a comparatively long period in the capillary bed, since the increase of histamine equivalent in the lymph lasts for an extremely long time. In any case, it seems safe to conclude that the majority of the vasodepressor effect by sinomenine is caused by histamine liberated in plasma and tissue fluid.

An intravenous injection of a mixture of sinomenine and dog blood, incubated at 38°C for 30 minutes, in a dog resulted in a long latent period prior to the depression of the blood pressure, as in the case of a simple injection of sinomenine alone, and the blood-drug mixture failed to cause any contraction of the guinea-pig's ileum. Histamine in the plasma, before and after injection of sinomenine, varied at a constant difference of approximately 0.1 µg/cc from that in the whole blood so that it is clear that this histamine does not originate from the formed element of blood (Table 3).
Incoagulability of the blood and lymph. In cases where sinomenine was sufficiently effective, blood and lymph became completely incoagulable. Such action was not witnessed when this drug was added to blood in vitro. The coagulation was finally induced in such blood and lymph a few minutes after the addition of a minute amount of toluidine blue which is known to cause the loss of anticoagulant activity by combining with heparin (17). In this case, the dye failed to show the same notable metachromatic color as when added to the pure heparin solution but still showed a similar coloration as in the case of plasma made incoagulable by peptone that it seemed possible to assume the presence of heparin.

**DISCUSSION**

It has been known that a number of widely different chemical compounds possess a common property of liberating histamine from mammalian tissues without any gross structural change. These compounds include some of the clinically important drugs such as D-tubocurarine (11, 18), morphine and other opium alkaloids (19, 20), pethidine, quinine, atropin (21), and trypanocidal diamidines (11). The histamine liberation by these drugs has recently been given attention in connection with the phenomena of the so-called drug allergy or drug idiosyncrasy such as giant edema, urticaria, and pruritus that are caused on the skin of human subjects susceptible to these drugs. In some histamine liberators, such as Compound 48/80 (22, 23) and propamidine (11, 20), the effects of the released histamine themselves account for most of their pharmacological actions. Such potent and specific histamine liberators are utilized as a useful means in studying various physiological and pathological problems regarding histamine liberation in tissues. However, no compounds are yet known whose histamine liberation can be utilized directly for therapeutic purposes. The evidences obtained with sinomenine, as described in the foregoing paragraphs, not only leaves no doubt as to the powerful and specific histamine releasing action of
this drug but also indicate clearly that the majority of the striking vascular effects caused by this drug, on which chief pharmacological actions including vasodepressor and lymphagogic effects found, are manifested through the liberated histamine. According to earlier workers (4,12), this action of sinomenine against peripheral circulation is thought as one of the most important mechanism of the striking antineuralgic effect of this drug.

There is still a room for doubt as to whether a similar clinical effectiveness can be assumed for other specific histamine liberators, since the susceptibility of tissue histamine in different organs may possibly vary with the kinds of the histamine releaser. There is still not enough data with the known compounds to fully discuss this point. However, as will be reported later, the present author has obtained a definite evidence that sinomenine possesses the property of releasing a large amount of histamine in the skin and muscles of a dog rather than in the liver which is recognized as the main site of action of other specific histamine liberators, such as peptone and certain organic bases (11) in this animal. This fact suggests that sinomenine is different from other compounds known as histamine liberators. Whether or not there is any relationship between the fact that sinomenine possesses a structure similar to that of opium alkaloids and that of histamine liberating action is of significance for separate studies.

Sinomenine shows various pharmacological properties that cannot be seen in histamine injection such as the facts that sinomenine itself has the action of depressing the gut and liberates an anticoagulant that is assumed to be heparin, and that the depression of arterial pressure and increase of protein concentration in the lymph caused by this drug cannot wholly be suppressed by antihistamines. However, these are not the chief pharmacological action of sinomenine.

SUMMARY

The present series of experiments clearly indicated that sinomenine, an antineuralgic drug, is a potent and specific histamine liberator, chief reasons for which are based on following evidences:

1. Intravenous injection of sinomenine in a dog causes depression of the arterial blood pressure, rise of portal pressure, increase in the volume of liver and hind limbs and accelerates flow of thoracic and cervical lymph. Intradermal injection of this drug, in men and dogs, causes a typical triple response. Striking tachyphylaxis is shown in any one of these actions and some of these actions are notably suppressed by antihistamine drugs.

2. Depression of arterial pressure by the intravenous injection of sinomenine requires a latent period of 20 to 50 seconds.
3. The presence of histamine, in an amount sufficient to support the action of this drug on blood vessels, is pharmacologically proved in the blood plasma directly after the injection of sinomenine. The amount of histamine in thoracic lymph shows a high value for a period longer than that in the blood plasma. The rate of histamine increase in the plasma and lymph is parallel to the degree of the vasodepressor and lymphagogic actions.

4. Coagulability of blood and lymph decreases or totally disappears after the injection of sinomenine, as in the case of other histamine liberators. This effect seems to be related to the liberation of heparin.

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REFERENCES

1) GOTO, K.: J. Japan. Chem. 3, Suppl. 2, 1 (1949)
3) ISHIWARI, N.: Ibid. No. 991, 767 (1921)
4) ISHIWARI, N.: Tokyo-Iji-Shinshi No. 2242, 1639 (1921)
6) YAMASAKI, H.: Ibid. 27, 35 (1939)
7) HEIDENHAIN, R.: Arch. ges. Physiol. 49, 209 (1891)
8) STARLING, E.H.: J. Physiol. 16, 224 (1894)
12) TAKAORI, S.: Chugai-Iji-Shimpo No. 996, 1106 (1921)
13) MAYEDA, H.: Folia pharmacol. japon. 48, 91§ (1952)
14) CODE, C.F.: J. Physiol. 89, 257 (1937)
15) BARSOUm, G.S. AND GADDDUM, J.H.: Ibid. 85, 1 (1935)
16) MCCARRELL, J.D.: Am. J. Physiol. 126, 20 (1939)
20) FELDBERG, W. AND PATON, W.D.M.: Ibid. 114, 490 (1951)
22) PATON, W.D.M.: Ibid. 6, 499 (1951)