The Effect of Hypertensin on the Inactivation of Oxytocin by the Serum of Pregnant Women

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A close relationship has been shown to exist between the enzymatic systems that inactivate hypertensin, oxytocin, and vasopressin. When cysteine or glutathione are added to tissue extracts (kidney), red blood cells, or plasma, the capacity of these preparations to inactivate the hormones of the neurohypophysis is increased in direct proportion to the content of hypertensinase (*2*).

Page (*5*) pointed out that oxytocinase differs from hypertensinase in its behavior during the course of normal pregnancy. Oxytocinase increases progressively, reaching by the last week of pregnancy a value 1,000 times that of the nonpregnant state; but the increase observed for hypertensinase, in the second half of pregnancy, is only 4–10 times the nonpregnant value.

TABLE 1*

Tubes	1	2	3	4	5	6
Oxytocin (units)	2	2	..	2	2	...
Hypertensin (units)	..	0.5	0.5	...	0.5	0.5
Pregnant serum (ml)	..	..	..	0.02	0.02	0.02

* The volume of each tube was made up to 2 ml with 0.9% NaCl after adding phosphate buffer, pH 7.3. Before testing, the tubes were incubated from 30 min to 8 hrs. Each solution, previously diluted, was assayed on isolated uterus.

Later, Croxatto, *et al.* (*3*), using as a test the effect on blood pressure in cats, showed that at the end of pregnancy the plasma hypertensinase level increases 3–4 times above that of the nonpregnant level, whereas vasopressinase exhibited an increase comparable to that observed for oxytocinase.

The experiments described in this paper indicate either that hypertensinase and oxytocinase have similar enzymatic properties or that there is a great similarity between the chemical structures of their substrates, since hypertensin interferes with the inactivation of oxytocin by the plasma of pregnant women.

Using the uterus of rats and guinea pigs to measure the oxytocinase activity of blood plasma, it was shown that the addition of hypertensin to a mixture of oxytocin and plasma retarded the rate of destruction of oxytocin. The different reagents were mixed in test tubes, the pH adjusted to 7.3 by means of a sodium phosphate buffer, and the mixtures incubated at 37° C for ½–8 hrs. Table 1 shows how the mixtures were prepared. The oxytocin used was a purified product which contained 10 uterotonic units/ml and an appreciable amount of vasopressin. The bath in which the uterus was submerged contained 60 ml of fluid, and the dilution of the mixtures for each tube varied, depending on the sensitivity of the uterus preparation. Fig. 1 shows some of the results obtained. The hypertensin in the doses employed showed no oxytocic effect. The retarding effect of hypertensin on the rate of inactivation of oxytocin is readily demonstrated, even with doses of less than 1 unit for every 2–5 uterotonic units of oxytocin.

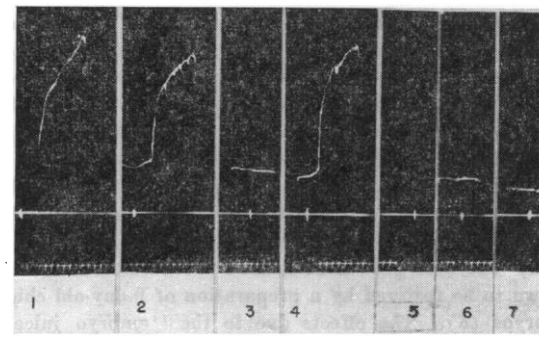

Fig. 1. Contractions of isolated guinea pig uterus. Additions: (1) 0.2 ml (dilution 1 : 60) of a mixture of 0.8 unit of oxytocin, 0.2 ml of sodium phosphate (pH 7.3), 1.35 ml of 0.9% NaCl in a total volume of 2 ml; (2) 0.2 ml (dilution 1 : 60) of 0.8 unit of oxytocin, 0.5 unit of hypertensin, 0.2 ml of serum 9th month of pregnancy; (3) same as (2) without hypertensin; (4) same as (2) without pregnancy serum; (5) same as (2) without oxytocin; (6) simultaneous addition of 3 and 5; (7) 0.2 ml of undiluted mixture added in (5).

The mixtures were incubated for 60 min at 37° C, buffered at pH 7.3 with sodium phosphate, and brought to a final volume of 2 ml with 0.9% NaCl.

In view of the fact that certain amino acids are capable of decreasing the rate of inactivation of oxytocin when used at high concentrations (*1*), the effect obtained in the above experiments might be attributed to amino acid impurities in the hypertensin solution. However, this is not likely because of the dilutions used and also because hypertensin, upon contact with plasma to which oxytocin has been added, loses its inhibitory effect on the oxytocinase after 8–10 hrs.

The possibility of a potentiating effect of hypertensin on oxytocin may also be eliminated, even though the former does increase slightly the uterotonic effect of oxytocin.

The inhibitory effect of hypertensin on oxytocinase slowly disappears as the incubation progresses, *i.e.* as hypertensin is destroyed.

The addition of cysteine or glutathione does not inhibit the retarding effect of hypertensin.

These results suggest that hypertensin competes with oxytocin as substrate for oxytocinase. Parallel experiments, using as a test the blood pressure in cats, have shown that the inactivation of vasopressin is also inhibited by hypertensin. If these effects are manifested *in vivo*, it may well be that the humoral mechanism which produces hypertensin indirectly interferes with the hormonal functions of the neurohypophysis.

The hypothesis that the hormones of the posterior

pituitary have a reciprocal influence on the inactivation of hypertensin is under study.

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The Effect of Browning on the Essential Amino Acid Content of Soy Globulin[1]

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Soybean oil meal turns brown during heat processing; in fact, the consumer has developed a preference for a brown-colored product with a cooked, nutty flavor. Although some heat treatment seems desirable, it is probable that a large share of the soybean meal on the market has been overheated (*3*). Soybeans contain about 30% carbohydrate, which apparently has never been completely characterized. A typical solvent-process meal will contain about 0.5% free reducing sugar, and an abundance of polysaccharides which can readily yield more reducing sugar. In addition, there are probably other browning reactants, which may be broadly described as compounds containing carbonyl radicals. Hence, soybeans constitute a system which is conducive to the occurrence of nonenzymatic browning (the Maillard reaction), and this reaction is probably responsible for the brown color which develops in the heat processing.

While there are probably several mechanisms which are responsible for the so-called "heat damage" to proteins, including the proteins in soybeans, we have been struck by the apparent similarity between the results recorded by Riesen, *et al.* (*6*), in which prolonged heating apparently destroyed part of the lysine, arginine, and tryptophan, and the results we obtained in studying the effects of the nonenzymatic browning reaction upon the nutritive value of casein (*5*). Consequently, we have determined the effect on the 10 essential amino acids of heating purified soy globulin in the presence of glucose.

A sample of commercial raw soybeans was finely ground and extracted with cold hexane. Soy globulin was then isolated by peptization with 10% NaCl and precipitated by dialysis. The process was repeated twice for further purification. The globulin was then washed with alcohol followed by ether, and dried at room temperature. Browned samples were obtained by refluxing 2.0-gm portions of the soy globulin for 24 hrs in 200 ml of 5% CP glucose and removing the glucose by dialysis before hydrolysis. In an attempt to eliminate heat *per se* as a factor, the control samples were refluxed for 24 hrs in 200 ml of distilled water. For tryptophan, refluxed samples were hydrolyzed for 24 hrs in 5N NaOH. For the other amino acids 30-hr hydrolysis in 6N HCl was used. The nutritive availability of the essential amino acids in the hydrolysates was determined microbiologically by the method of Stokes, *et al.* (*7*), modified by substituting sucrose for glucose in the media to prevent further browning loss (*4*). *Lactobacillus delbrückii* LD5 was used for phenylalanine; *Streptococcus faecalis* R, for the other amino acids. The final concentration of sucrose in the media was 19 gm/liter; both organisms grew well in sucrose instead of glucose. The results are shown in Table 1. These indicate that glucose

TABLE 1

Essential Amino Acid Content of Soy Globulin Refluxed 24 Hrs

Amino acid	In water %	In 5% glucose %	Loss in glucose %
Lysine	5.84	4.24	27.4
Arginine	3.43	2.65	22.7
Tryptophan	1.44	1.22	15.3
Histidine	3.21	2.74	14.6
Methionine	1.20	1.24	−3.3
Leucine	7.62	7.53	1.2
Isoleucine	6.28	6.37	−1.4
Valine	5.41	5.23	3.3
Threonine	3.94	3.83	2.8
Phenylalanine	5.10	5.15	−1.0

interaction destroyed 27.4% of the lysine present, 22.7% of the arginine, 15.3% of the tryptophan, and 14.6% of the histidine. It is doubtful if any of the other essential amino acids were affected.

Under the conditions of this experiment, the use of a water-refluxed control turned out to be unnecessary. Apparently the destruction observed was entirely due to the glucose and not to the heat *per se*, since comparison of the total essential amino acid content of untreated and water-refluxed samples showed the presence of 43.49% and 43.47%, respectively.

The opinion has been expressed in Quartermaster Reports and elsewhere that a toxic, growth-inhibiting substance is produced as a result of the browning reaction. In such a case, one would expect to obtain decreased growth in each of the amino acid assays. Since in our experiments certain amino acid assays gave equal growth responses before and after browning, it seems unlikely that a toxic substance is involved.

Whether the amino acids are nutritionally less available but still chemically present is a disputed point.

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