Effect of Human Pregnancy Serum on Avian Depressor Activities of Oxytocin and Desamino-Oxytocin.* (28293)

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In a previous report(1) from this laboratory, the susceptibility of oxytocin and a highly active analogue of oxytocin, desamino-oxytocin(2-5), to attack by hog kidney leucine aminopeptidase and α-chymotrypsin was investigated by measurement of their avian depressor activity before and after incubation with the enzymes. Desamino-oxytocin was not inactivated by leucine aminopeptidase under conditions in which 50% of the activity of oxytocin was lost(1). On the other hand, desamino-oxytocin was found to be completely inactivated by α-chymotrypsin under conditions in which some of the activity of oxytocin still remained(1). As a continuation of these studies, the effects of human pregnancy and non-pregnancy sera on the avian depressor activities of oxytocin and desamino-oxytocin were investigated. Human pregnancy serum (or plasma) has been reported to contain oxytocinase and the inactivation of biological activity of oxytocin was attributed to this enzyme(6-10). Oxytocinase was found to be absent in plasma of pregnant rabbits, rats, guinea pigs, hogs, horses, cows, dogs, and cats but was present in the plasma of a pregnant rhesus monkey(6-9). In addition to attacking oxytocin, pregnancy serum was also found to hydrolyze a synthetic substrate, L-cystine-di-β-naphthylamide (10-12), and several S-benzylcysteine peptides(13), at rates greater than those found with non-pregnancy serum.

Materials and methods. Serum. Non-pregnancy serum was obtained from the blood of adult non-pregnant female laboratory personnel. Pregnancy serum was obtained from blood collected from obstetrical patients undergoing active labor. The blood samples were obtained from the arm veins. The serum was stored in the frozen state and showed no diminution in activity after storage for several weeks. *L-Cystine-di-β-naphthylamide assays.* L-Cystine-di-β-naphthylamide was synthesized according to the method of Tuppy and Nesvadba(12,14). The hydrolysis of this substrate was determined following the method of Müller-Hartburg, Nesvadba, and Tuppy using a Bratton-Marshall procedure for estimation of liberated β-naphthylamine(11,12). The results were expressed as mg of β-naphthylamine liberated per hr per 100 ml of serum. *Oxytocin and desamino-oxytocin inactivation experiments.* Oxytocin or desamino-oxytocin was treated with pregnancy or non-pregnancy serum in the following manner. One ml of serum was added to a mixture consisting of 1.0 ml of 0.046 M veronal buffer, pH 7.9, 1.0 ml of an aqueous solution of oxytocin or desamino-oxytocin containing 20 units of avian depressor activity per ml, and 1.0 ml of water. Immediately after addition of serum, the mixture was mixed rapidly and a 2 ml aliquot was removed, heated in a boiling water bath for 2 minutes, cooled in an ice bath, and then frozen. This was used as the zero time sample. The remainder of the digestion solution was allowed to incubate at 37°C for 4 hours before the enzyme activity was stopped as described for the zero time sample. Avian depressor assays were carried out on chickens according to the procedure of Munsick, Sawyer, and van Dyke(15). The final digestion solutions were assayed directly against the zero time samples. The activity in the final digestion solutions was expressed as percent of initial activity present in the zero time samples.

Results and discussion. The results obtained on the hydrolysis of L-cystine-di-β-naphthylamide and the effects of human pregnancy and non-pregnancy sera on the avian depressor activities of oxytocin and desamino-oxytocin are shown in Table I. The data of this Table demonstrate that non-

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TABLE I.

Serum	L-Cystine-di-β-naphthylamide splitting activity (mg of β-naphthylamine liberated per hr per 100 ml of serum)	% of initial avian depressor activity after treatment with serum	
		Oxytocin as substrate	Desamino-oxytocin as substrate
Non-pregnant	.19	99	102
"	.19	101	95
Pregnant	5.2	1	103
"	4.6	2	99

pregnancy serum has a low L-cystine-di-β-naphthylamide-splitting activity and was without effect on the avian depressor activities of oxytocin and desamino-oxytocin. On the other hand, pregnancy serum was at least 24 times more active in splitting L-cystine-di-β-naphthylamide than was non-pregnancy serum. Moreover, pregnancy serum almost completely inactivated the avian depressor activity of oxytocin but was without effect on desamino-oxytocin.

Our results on the hydrolysis of L-cystine-di-β-naphthylamide by pregnancy and non-pregnancy sera agree very well with results obtained by others. Müller-Hartburg, Nesvadba, and Tuppy obtained mean values of 0.20 and 5.60 mg of β-naphthylamine liberated per hr per 100 ml of serum, respectively, for non-pregnancy and pregnancy serum (11) while Riad(16) and Titus *et al.*(17) obtained values of 0.30 and 4.8, and 0.2 and 5.0, respectively.

Other workers have considered serum oxytocinase to be an aminopeptidase(10,13). Tuppy and Nesvadba found that serum oxytocinase split the hemicystinyltyrosine bond in the pentapeptide ring of oxytocin thus cleaving the ring and concluded that the enzyme was an aminopeptidase(12). Berán-ková, Rychlík, and Sorm found that pregnancy serum was able to hydrolyze S-benzyl-cysteinyltyrosine but was unable to hydrolyze carbobenzoxy-S-benzylcysteinyltyrosine (13). The present study provides initial data on the effect of pregnancy serum on an analogue of oxytocin, namely, desamino-oxytocin, which has no free amino group and yet possesses higher potencies than oxytocin in some of its biological activities(3-5). The lack of a free amino group in desamino-oxytocin renders the compound resistant to attack by pregnancy serum. Thus, the data obtained support the view that serum oxytocinase behaves as a typical aminopeptidase.

Summary. The effects of human pregnancy and non-pregnancy sera on the avian depressor activities of oxytocin and desamino-oxytocin have been investigated. Pregnancy serum had no effect on desamino-oxytocin under conditions in which oxytocin was inactivated. Non-pregnancy serum was without effect on either compound.

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Some Effects of Cold-Acclimation on the Biochemistry and Histology of the Hamster Kidney.* (28294)

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The striking enlargement of the kidney which accompanies cold-acclimation in the hamster, *Mesocricetus auratus*(1), suggests that this organ may play a particularly important compensatory role in maintenance of thermal homeostasis. Since growth reflects cellular changes, a basic understanding of the nature of this renal enlargement must await considerable histological, cytological, and biochemical information. Studies on this subject have not been reported by other workers, and we have reported our results only briefly (2,3). This communication documents these preliminary notes, and presents in detail other studies not previously reported. It includes (a) a histological analysis of the early and advanced stages of acclimation, (b) assays of total renal DNA, protein, and microsomal nitrogen, (c) assays of microsomal DPNH-cytochrome c reductase, and (d) assays of succinoxidase, succinic dehydrogenase, and transaminase of kidney homogenates. Body and kidney weights of both heat- and cold-acclimated male hamsters, and of cold-acclimated females are also presented.

Methods and materials. Adult hamsters weighing from 80 to 140 g were caged singly and given Purina chow pellets and water *ad libitum*. A bed of wood chips was placed in each cage. Throughout each study involving measurement of the same parameter, the following factors were constants: 1) age limits of animals used, 2) temperature of control room, 3) temperature of acclimation room, and 4) duration of acclimation period. In many of the studies some of these factors were altered, and these details are given with tabulated results in Tables I-V. Males were used exclusively, except in one study designed to compare cold-induced kidney growth in male and female hamsters.

At the end of the acclimation period, the animals were weighed, killed by decapitation, and bled. The kidneys were decapsulated, rinsed in cold tap water, rolled against damp filter paper, and weighed immediately. They were then minced and expressed through an ice-cold fine-grille stainless steel tissue press, and the resulting pulp homogenized in a Potter-Elvehjem iced tissue grinder. Tissues for DNA and total nitrogen assays were homogenized in distilled water. Isolation of microsomes was carried out in 8.5% sucrose

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