Effect of Pitressin® Infusion on Plasma Free Fatty Acid Levels in Man. (29524)

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During studies on the response of the sympathetic nervous system to water immersion, it was noted that several subjects experienced orthostatic hypotension and a fall in plasma free fatty acid (FFA) levels during a passive tilt in contrast to the more usual increase in diastolic blood pressure and the concentration of plasma FFA(1). Concurrently, the subjects exhibited antidiuresis, epigastric distress, ashen facies, restlessness, weakness, feelings of heat, palpitation and diaphoresis, such as have been noted after infusion of antidiuretic hormone(2). The recent demonstration that vasopressin reduces the concentration of FFA in the plasma of dogs(3) prompted the present study on the effect of vasopressin infusion on plasma FFA concentrations in man.

Methods. Five healthy young men served as subjects in this study. They were fasted for 10 hours, but water was allowed *ad lib.* At 8 a.m. each was weighed and assumed the supine position. A number 20 needle was placed in an antecubital vein and connected by a 2-inch length of polyethylene 50 tubing to a three-way stopcock connected in turn to 2 tuberculin syringes in separate constant infusion pumps. One syringe contained isotonic saline and the other Pitressin® (Parke-Davis, USP), a posterior pituitary preparation containing both arginine and lysine vasopressins (20 units/ml) and oxytocin (1.1 units/ml) which was diluted with isotonic saline to 1 pressor unit per milliliter. Both pumps were set at identical rates of infusion, determined by the subject's weight, so as to deliver 0.01 pressor units of Pitressin® per kilogram per hour.

Saline infusion was started and blood samples were drawn at 20, 40, and 60 minutes. After one hour the stopcock was turned, the Pitressin® infusion started, and blood samples taken at 10-minute intervals for the next hour. The stopcock was then returned to its original position and saline was infused for another hour during which blood samples were taken every 20 minutes.

The blood samples (10 ml in each instance) were drawn through a number 20 needle placed in the contralateral antecubital vein and kept patent with a slow saline drip. Oxalate tubes were used for blood collections. Immediately after withdrawal, each sample was mixed and an aliquot removed and frozen for subsequent glucose analysis by the ferricyanide autoanalyzer technique (4). The remainder of the sample was quickly centrifuged and the plasma removed and frozen for subsequent determination of the FFA concentration by Trout's modification(5) of the Dole procedure(6). Mean values of concentrations of glucose and FFA in the 3 samples obtained from each individual during the initial period of saline infusion are designated as 100% for that subject. All subsequent values are reported as per cent of these "mean baseline" levels.

Results. The plasma FFA concentrations in microequivalents per liter are presented for each subject at each time period in Table I. The average per cent ($\pm$ SE) of "mean baseline" for each time period for all subjects is depicted in Fig. 1.

The initial effect of Pitressin® infusion was a statistically insignificant ($0.1 > p > 0.05$) increase in FFA concentration. After 40 minutes of infusion, however, a statistically significant ($p<0.05$) decrease became apparent. This decrease progressed until Pitressin® was discontinued after which FFA concentration rapidly returned to baseline.

Blood glucose concentration did not change significantly at any time during the experiment.

Discussion. The data reported herein reveal that the effect of Pitressin® on plasma concentration of FFA in man is essentially

TABLE I. Plasma FFA Levels (mEq/l) Before, During, and After Pitressin® Infusion.

	Saline			Pitressin®						Saline		
Time	20	40	60	10	20	30	40	50	60	20	40	60
Subject I	519	534	505	635	650	534	490	447	375	403	447	461
II	375	457	456	481	424	400	391	366	358	457	456	480
III	543	521	506	521	484	521	484	455	425	433	477	462
IV	552	756	688	777	701	762	688	579	579	715	708	715
V	308	295	268	268	362	228	188	—	241	268	247	275

the same as that observed in male dogs given the same dose of vasopressin(3). The initial rise noted in man but not in dogs is statistically insignificant and may be artifactual. An infusion of 0.0067 pressor units per kilogram was required to produce a significant fall in plasma FFA's in man, whereas dogs responded similarly to 0.0033 pressor units per kilogram. This difference in sensitivity may be attributed to species characteristics as observed *in vitro* by Rudman(7).

The mechanism by which Pitressin® lowers plasma FFA concentration in man is unknown. Since vasopressin can cause ACTH release in some animals(8,9) and the latter can lower circulating FFA in man(10), it is possible that the effect of vasopressin noted in this study is mediated through endogenous ACTH. This seems unlikely, however, since the release of ACTH in man requires 50 to 200 mU vasopressin per kilogram(9) instead of 6.7 mU per kilogram which produced a significant decrease in the FFA's in this study.

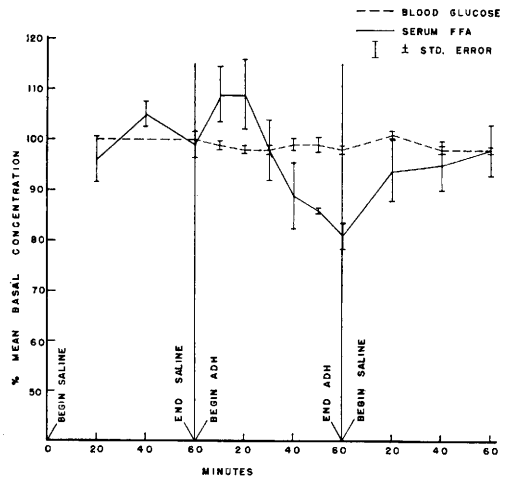

FIG. 1. Mean responses of plasma FFA and blood glucose to an intravenous infusion of vasopressin (0.01 μ/kg/hr). Vertical lines indicate ± 1 standard error.

Vasopressin inhibits the degradation of insulin by liver extracts(11) and may therefore increase amounts of circulating active insulin which would in turn lower plasma FFA concentrations. The absence of any change in blood glucose concentrations makes this unlikely but further studies in man are required in view of the recent demonstration that insulin can suppress release of free fatty acids from excised adipose tissue without affecting glucose utilization(12). An insulin mediated mechanism has been ruled out in dogs(3) by the use of alloxan diabetic animals, but such proof in man requires measurement of plasma insulin-like activity before, during and after vasopressin infusion.

Summary. The infusion of 0.01 pressor units Pitressin® per kilogram per hour produced a decrease in plasma FFA concentrations in healthy young men without changing blood glucose concentration. The effect was evident after 40 minutes of infusion and progressed to a value equal to 80% of baseline in one hour. These findings support the hypothesis that vasopressin may have contributed to the decreasing plasma concentrations of FFA of man noted concurrently with hypotension during a passive tilt after water immersion.

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In vivo Activity of Colloidal Hamycin, an Antifungal Antibiotic. (29525)

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Hamycin is a polyene antifungal antibiotic obtained from *Streptomyces pimprina*. The antibiotic has been studied intensively in India, where it is produced, but has received scant attention in the U.S.A. Hamycin given orally or intraperitoneally to mice has been able to eradicate infection due to *Cryptococcus neoformans*(1). The drug is active *in vitro* against *Candida albicans* and has been used successfully to treat thrush in man(2).

Hamycin is insoluble in water. Experience with another water-insoluble polyene, amphotericin B, has indicated that a colloidal dispersion of the drug was easier to dispense, was more active *in vivo*, and probably persisted longer in the circulation than the crystalline form(3). For this reason, a colloidal dispersion of hamycin was prepared, bioassayed, and its chemotherapeutic activity assessed in mice infected with *Cryptococcus neoformans* and *Histoplasma capsulatum*. Acute toxicity of the preparation was assessed in mice and rabbits.

Materials and methods. Unnumbered preparations of hamycin were received 1/22/63 and 10/29/63. They will be referred to as numbers 1 and 2 respectively. The yellow powders were dried *in vacuo* at 75-100 μ Hg for 6 hours and stored at 4°C. Solutions were prepared as 2 mg/ml in dimethyl sulfoxide (DMS). For bioassay, fresh DMS solutions were diluted in phosphate buffered saline pH 7.4 (PBS) to concentrations of 0.04-0.32 μg/ml. To determine absorption coefficients, DMS solutions were diluted 300-fold in a solvent containing 3 volumes ethanol and 2 volumes distilled water. Absorption maxima as measured in a Beckman DU spectrophotometer were 364, 384 and 408 mμ. Absorption coefficients were expressed as $E^{1\%}_{1\,cm}$ at $\lambda = 384$ mμ.

A colloidal dispersion of hamycin #1 was prepared by a method similar to that described for amphotericin B(4). Hamycin 2.42 g and sodium desoxycholate 3.1 g were added to 375 ml distilled water and stirred in an ice bath until the suspension reached 7°C. Addition of 8.2 ml N sodium hydroxide resulted in a pH rise to 12.6. After several minutes of stirring, the solution became nearly clear. It was then neutralized with N hydrocloric acid. Centrifugation at 5°C removed 20 mg sediment. The optically clear yellow supernate was divided into portions, frozen in alcohol-dry ice, and stored at —42°C. A portion was lyophilized, revealing a dry weight of 15.21 mg per ml solution.

On 2 occasions, colloidal hamycin solutions were thawed, divided into 3 ml samples, and quickly returned to —42°C storage. One sample on both occasions was not refrozen but bioassayed. A similar operation was per-