Effect of Arginine-Vasopressin and Lysine-Vasopressin on Plasma 17-Hydroxycorticosteroid Levels in Man. (22518)

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It was previously reported(1) that intravenous infusion of commercial vasopressin (Pitressin) in man causes an increase in the concentration of free 17-hydroxycorticosteroids (17-OHCS) in plasma. Because of the presence of certain unidentified substances in Pitressin reported to cause ACTH release (2,3), it was deemed necessary to study the effect of highly purified vasopressin on plasma 17-OHCS. Two observations are pertinent to this consideration: Guillemin and Hearn showed that Pitressin added to rat anterior pituitary glands in organ culture caused a release of ACTH, whereas highly purified arginine-vasopressin did not(4). On the other hand, Sayers demonstrated that highly purified arginine-vasopressin has the same ACTH-releasing activity as Pitressin in the adrenalectomized rat(5).

Because preliminary observations indicated that injection of arginine-vasopressin caused a smaller increase in plasma 17-OHCS than did Pitressin, the method of preparation of the latter was investigated. It was learned that Pitressin contains arginine-vasopressin and lysine-vasopressin, since both bovine and porcine posterior pituitary lobes are utilized. This observation suggested the possibility that lysine-vasopressin might cause a greater increase in plasma 17-OHCS than arginine-vasopressin. Accordingly, we conducted comparative studies on these 2 compounds.†

Materials and methods. Twelve normal male subjects, ranging from 18 to 31 years of age, were studied. *Test solutions.* (1) 0.9% saline; (2) vasopressin diluent [0.5% chlorobutanol solution adjusted to pH 3.6 with acetic acid]; (3) vasopressin in the above diluent; and (4) Pitressin [Parke, Davis & Co., Lot 909E] in the above diluent. All tests were begun at 8 a. m. with the subjects remaining in bed in a post-absorptive state. Intravenous infusions were given at the rate of 3 ml per minute and consisted of approximately 180 ml of saline plus 4.2 ml of vasopressin plus diluent or vasopressin diluent alone. Injections were given over a period of 1 minute in a volume of approximately 2 ml. *Blood samples.* Venous blood samples of 20 ml were drawn through an indwelling Cournand needle immediately prior to administration of test solution and at intervals thereafter. The heparinized blood was centrifuged and plasma removed and frozen until time of determination. Concentration of free 17-OHCS in the plasma was determined by the method of Silber and Porter(6), as modified by Peterson, *et al.*(7). Duplicate determinations were obtained on all plasma samples. *Experimental design.* The first part of the study was an evaluation of the effect of intravenous infusion of arginine-vasopressin on plasma 17-OHCS. Each of 5 subjects received a one-hour infusion of saline plus vasopressin diluent either preceded or followed by a one-hour infusion of saline plus arginine-vasopressin. On another day the order in which test solutions were administered was reversed. Blood samples were drawn immediately before, and at 30, 60, 90, and 120 minutes after the infusion was begun. The second part of the study was an evaluation of the effect of intravenous injections of Pitressin, arginine-vasopressin, and lysine-vasopressin on plasma 17-OHCS. Four subjects were given 2 units of Pitressin on 1 day and a comparable volume of vasopressin diluent on another day. Eight subjects were given 2 units of arginine-vasopressin on 1 day and 2 units of lysine-vasopressin on another day. The order of administration of test solutions was

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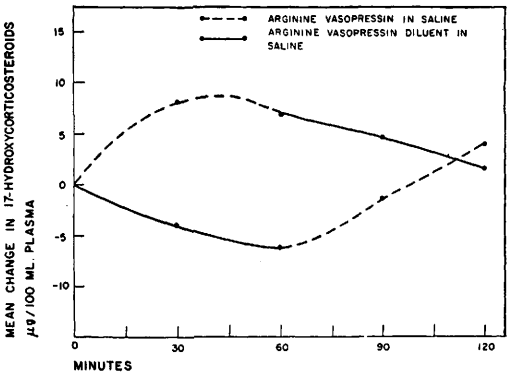

FIG. 1. Effect of arginine-vasopressin on plasma 17-hydroxycorticosteroid concentration. Individual points represent mean values for a group of 5 young, normal subjects receiving 12 to 16 units of arginine-vasopressin in saline in one-hr infusion either preceded or followed by one-hr infusion of saline plus arginine-vasopressin diluent.

randomized in both groups. Blood samples were drawn immediately before, and 15, 30, and 60 minutes after injection.

Results. The pretest levels of plasma 17-OHCS based on the 34 initial values obtained in this study averaged 16.4 μg % with a standard deviation of 5 μg %.

Infusion of Arginine-Vasopressin. The effect of intravenous infusion of arginine-vasopressin is shown in Fig. 1. Plasma 17-OHCS levels associated with infusion of arginine-vasopressin are significantly higher than those associated with infusion of vasopressin diluent. Increases observed are comparable in degree to those associated with infusion of similar amounts of Pitressin. The degree of nausea, abdominal cramping, and peripheral vasoconstriction experienced by individuals receiving infused arginine-vasopressin was similar to that noted with infusion of Pitressin(1).

Intravenous injection of Pitressin and vasopressin diluent. Injection of identical volumes of Pitressin and vasopressin diluent was undertaken to determine their effects on plasma 17-OHCS. It is apparent from Fig. 2 that injection of 2 units of Pitressin causes a rise in plasma 17-OHCS, whereas no rise occurs with injection of vasopressin diluent. The rise associated with injection of Pitressin is significant at 30 minutes ($P<0.01$) and is probably significant at 15 and 60 minutes ($P< 0.05$).

Intravenous injection of arginine-vasopressin and lysine-vasopressin. Both arginine-vasopressin and lysine-vasopressin cause an increase in plasma 17-OHCS in this experimental situation. As shown in Fig. 2, the increase associated with intravenous injection of 2 units of lysine-vasopressin is more than twice that observed when 2 units of arginine-vasopressin are given in the same manner. The different degree of response following injection of these 2 substances is probably real ($P<0.02$ at 30 minutes; $P<0.05$ at 15 and 60 minutes).

Summary. (1) Intravenous administration of highly purified arginine-vasopressin in man is associated with a significant increase in plasma concentration of free 17-OHCS.

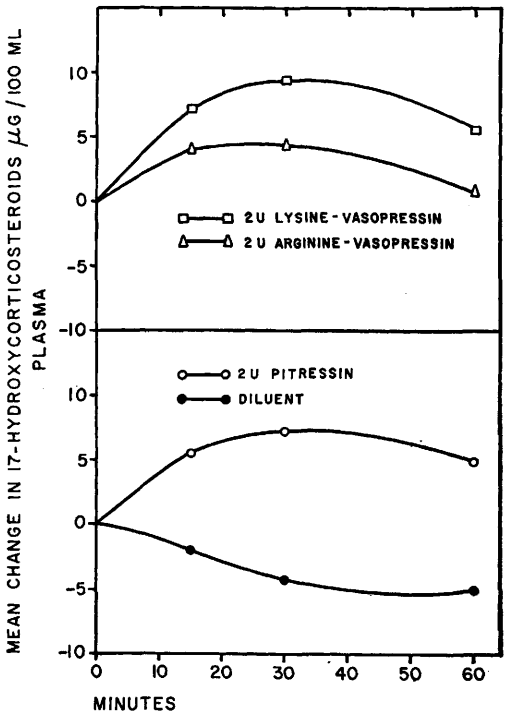

FIG. 2. Effect of intravenous injection of 2 U Pitressin, lysine-vasopressin, arginine-vasopressin, or equivalent vol of diluent, on plasma 17-OHCS concentrations. *Upper graph,* mean values of 8 young, normal males receiving, on different days, lysine-vasopressin and arginine-vasopressin. *Lower graph,* mean values of 4 young, normal males receiving, on different days, Pitressin and vasopressin diluent.

(2) Highly purified lysine-vasopressin causes higher plasma 17-OHCS levels than highly purified arginine-vasopressin when administered by means of an intravenous injection. (3) Increase in plasma concentration of free 17-OHCS observed when Pitressin is administered in man can be completely accounted for by the vasopressin content of Pitressin.

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Nithiazide I. Chemical and Biological Studies on 1-ethyl-3-(5-nitro-2-thiazolyl) urea and Related Compounds. (22519)

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Enterohepatitis (blackhead disease) may be an important cause of morbidity and mortality in turkey flocks. Although a number of substituted 8-hydroxyquinolines and arsenical compounds(1,2) were known to have some value in enterohepatitis, the discovery of the effectiveness of 2-amino-5-nitrothiazole by Waletzky *et al.*(3) revealed an important new chemical means of controlling this disease. During the course of screening various substituted ureas(4), we found that 1-ethyl-3-(5-nitro-2-thiazolyl) urea was effective in controlling the morbidity and mortality of enterohepatitis. This compound has been assigned the generic name of nithiazide. The present paper reports the results of the chemical and biological studies on nithiazide and related compounds.

Materials and methods. Chemical. The 1-mono-substituted nitrothiazolylureas employed in this study were prepared by the addition of various isocyanates to 2-amino-5-nitrothiazole. The preparation of 1-ethyl-3-(5-nitro-2-thiazolyl) urea (Example 1) is representative of this general method. Synthesis of 1,1-disubstituted nitrothiazolylureas was conveniently effected by heating 2-amino-5-nitrothiazole and a disubstituted carbamyl chloride in an inert solvent (Example 2). The basicity of 2-amino-5-nitrothiazole is sufficiently feeble that the hydrogen chloride produced in this reaction is readily expelled by the refluxing solvent. *Example 1. 1-Ethyl-3-(5-nitro-2-thiazolyl) urea.* To a solution of 69 g (0.97 mole) of ethyl isocyanate and 1200 ml of dry toluene was added 94 g (0.648 mole) of 2-amino-5-nitrothiazole. The stirred suspension was heated 16 hours at vigorous reflux under scrupulously anhydrous conditions. The warm slurry (80°) was filtered, and the pale yellow crystalline cake of 1-ethyl-3-(5-nitro-2-thiazolyl) urea was washed well with benzene and ether, and then dried to constant weight. Yield: 137.7 g (98.5%); m.p. 228° (dec.) Anal. Calcd. for $C_6H_8O_3N_4S$: C, 33.33; H, 3.73; N, 25.91. Found: C, 33.70; H, 3.58; N, 25.44. Infrared spectrum (Nujol mull): Intense carbonyl band at 5.95 μ; broad N-H absorption at 3.2 μ. Ultraviolet spectrum (ethanolic hydrochloric acid): $\lambda_{max.}$ 350 mμ $\epsilon_{max.}$ 14910; $\lambda_{max.}$ 234 mμ, $\epsilon_{max.}$ 7890. *Example 2. 1,1-*