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Stimulation of ACTH-Release in Humans by Non-Pressor Fraction from Commercial Extracts of Posterior Pituitary.* (23605)

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Isolation of a purified material of hypothalamic origin which stimulates release of ACTH *in vitro* was recently reported from this laboratory(1). Similar activity was found in the corresponding fraction when commercial extracts of posterior lobe of the pituitary were subjected to the same fractionation procedures. Evidence has been obtained that the active material is a small peptide different from vasopressin and oxytocin (1). The physiological significance of these findings as well as the composition and structure of the active factor are still to be elucidated. Even at this stage of fragmentary knowledge, it was of interest to investigate whether or not the hypophysiotropic fraction would stimulate the release of endogenous ACTH in humans. Stimulation of ACTH-release in the human has never been reported in the absence of stressing conditions such as fever, pain, discomfort, surgical procedures, electroshock, insulin shock, forced muscular exercise or emotional upset. The active hypophysiotropic material appeared devoid of any side effects on the basis of pharmacological studies(1) and produced increases of the plasma 17 hydroxycorticoids when administered to dogs(2,†). It was therefore decided to study its possible ACTH-releasing activity in humans without regard for the explanatory mechanism, *i.e.*, directly hypophyseal (specific) or trans-hypothalamic (non-specific), of any positive result as long as it would have been obtained in the absence of stress or general discomfort.

Material and methods. Two types of clinical investigations were carried out in a total of 36 human subjects. a. 5 to 10 mg of a material obtained by chromatographic fractionation of oxycellulose-washed Protopituitrin‡ and designated as fraction D(1), were administered by I.V. perfusion in 150 to 200 ml of saline over 4 hours to 14 hospitalized children (age 4 to 16 years) convalescent from various conditions. Determinations of the plasma "free" 17OH-corticoids (17-OHC) were done by a modification of the method of Nelson and Samuels(3) at times 0, 2, 4, 6 hours as a test of stimulation of ACTH-release. In some of these studies measurements of the urinary excretion of 17OHC by the method of Glenn and Nelson(4) were

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‡ Protopituitrin (Parke, Davis & Co.) is a crude acetic acid extract of post. pituitary (fraction C in Waring & Landgrebe's outline of Kamm fractionation procedure(7)). It is the commercial starting material for Pitressin® and Pitocin®.

TABLE I. Absolute Values and Variations of Concentration of Free Plasma 17OH-Corticoids in Subjects Receiving a 4-Hour Perfusion of Fraction D or an Identical Perfusion with Saline or Inactivated Material as Control.

	Time	0	2 hr	4 hr	6 hr
Fraction D	17OHC, μg %	12.7 ± 1.5	13.7 ± 2.0	27.1 ± 5.5	21.3 ± 12.9
	17OHC variations		+.1 ± 3.4	+14.4 ± 4.5	+9.3 ± 13.0
	No. of subjects	14	7	14	7
Saline controls or inactivated material	17OHC, μg %	18.2 ± 2.6	11.1 ± 4.1	10.5 ± 2.1	
	17OHC variations		−7.0 ± 3.8	−7.7 ± 3.5	
	No. of subjects	8	8	8	

carried out on aliquots of 24-hour urine specimens. Control studies were run on 8 patients with similar perfusions of saline or with inactivated fraction D (by alkaline hydrolysis). All perfusions were carried out between 7-8 a.m. to 11-12 a.m. The hypophysiotropic activity of the various batches of fraction D utilized in these studies was assessed *in vitro* (1) prior to administration. In all the 4-hour perfusion studies, the blood pressure was measured every 15 minutes by indirect sphygmomanometry for the duration of the infusion. Pulse, respiration, body temperature were checked and recorded as well as any subjective sign suspected or reported by the subjects. b. 25 to 45 μg of electrophoretically purified fraction DΔ(1) in 1 or 2 ml saline were administered in one single rapid I.V. injection to 5 adult healthy male subjects. Plasma free 17OHC concentrations were measured at time 0, 15, 30, 60 minutes. Simultaneous control studies were run with inactivated fraction DΔ. All materials were assayed *in vitro*(1) for stimulation of ACTH-release. By chromatography, electrophoresis and *in vitro* assays(1), fractions D and DΔ appear to have no ACTH contamination or activity. *In vivo,* fraction D (up to 1.5 mg) shows no ACTH activity by Sayers test in rats of 150-180 g or when injected (1 mg) in hypophysectomized dogs with measurement of adrenal vein 17OHC secretion(2). After adequate priming of the adrenal cortex with ACTH showing that the adrenals were responsive to stimulation, 20 mg of fraction D dissolved in 16% Armour USP gelatin solvent were given (5 mg, q-12 hr, 4x) to a patient with panhypopituitarism: urinary and plasma 17OHC levels fell abruptly during administration of this large dose of fraction D. Thus it can be reasonably ascertained that fraction D and DΔ have no ACTH activity or contamination at doses comparable or larger than those utilized here.

Results. A statistically significant elevation of the plasma 17OHC was obtained after 4 hours of perfusion with fraction D (Table I). Large variations in responses were observed. In spite of these variations even the *absolute* values of the plasma 17OHC at 4 hours are statistically different from those at time zero ($p \leq 0.01$). It is possible that the 6 hour reading may indicate a residual stimulation of the pituitary-adrenal system if correlated with the corresponding values in the diurnal decline. A marked increase in the excretion of urinary 17OHC was observed in the 24-hour period starting with the administration of fraction D in 3 cases studied.

The rapid I.V. injection of fraction DΔ resulted in an increase of the plasma 17OHC after 15 minutes, with a gradual decline over 60 minutes. Due to the small sampling of the group receiving active material, this elevation is at the limit of statistical significance when compared against 17OHC concentration at time 0 in the same group. The observed increase is however statistically significant ($0.02<p<0.05$) when compared with the corresponding 15 minute values of the control group.

No objective changes were observed in the blood pressure, pulse, pulse pressure, cornea or skin color, skin moisture, respiratory rate or body temperature with any of the preparations administered. None of the subjects reported any feeling of general discomfort, nausea, intestinal cramp, or headache. The only remark sometimes made by the subjects

TABLE II. Absolute Values and Variations of Concentration of Free Plasma 17OH-Corticoids in Subjects Receiving One Intravenous Injection of 25-45 μg of Fraction DΔ or an Identical Amount of Inactivated Material as Control.

	Time	0	15 min.	30 min.	60 min.
Fraction DΔ	17OHC, μg %	10.6 ± 2.7	16.8 ± 3.3	14.1 ± 6.0	12.0 ± 3.2
	17OHC variations		+6.2 ± 2.7	+4.5 ± 3.4	+1.4 ± 2.4
	No. of subjects	5	5	4	5
Controls, inactivated material	17OHC, μg %	11.9 ± 2.4	9.0 ± 1.5	8.9 ± 1.6	7.7 ± 1.7
	17OHC variations		−2.9 ± 1.1	−3.0 ± 1.5	−4.2 ± 1.0
	No. of subjects	9	9	9	9

receiving the acute injection of DΔ (in either active or inactivated form) was of a very mild sensation of warmth in the face lasting for a few seconds. Subsequently it was observed in the dog under Nembutal anesthesia with direct recording of the blood pressure through the carotid artery that rapid I.V. injections of 5 to 10 times the amount of DΔ given to the patients would produce a transient fall in carotid pressure. At the doses of DΔ given to the humans the effect was absent or non-significant. This transient vasodilatory effect was observed in the dog with active or inactivated DΔ and would appear to be due to an otherwise inactive contaminant.

Discussion. The limited number of experiments carried out with fraction DΔ was due to the conditions of its availability. So far the hypophysiotropic material is still made by paper chromatography and subsequent paper electrophoresis(1). The yields are extremely low and the reported lability of the electrophoretically purified fraction (as followed by the *in vitro* assay) has not been explained nor adequately controlled. Other methods of preparation of the highly purified fraction are being investigated. In this limited series of investigations a complete correlation of *in vitro* and *in vivo* results was obtained with both types of materials utilized. In contradistinction to the reports of McDonald *et al.*(5,6) these results show that stimulation of endogenous release of ACTH in humans as evidenced by a test based on the measurement of plasma corticoid concentrations can be obtained in the absence of painful side effects with a material extracted from Protopituitrin and devoid of pressor activity at the doses utilized here.

Summary. A non-pressor material, extracted from Protopituitrin, known to stimulate release of ACTH *in vitro* was shown to stimulate release of ACTH in human subjects as evidenced by measurements of plasma 17-hydroxycorticoids. This increased secretion of ACTH was obtained in absence of significant side effects.

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