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Sequence of pituitary–adrenal cortical hormone responses to low-dose physostigmine administration in young adult women and men

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Abstract

We previously demonstrated greater HPA axis activation in adult men compared to adult women following low-dose administration of the anticholinesterase inhibitor, physostigmine (PHYSO). Because blood sampling was done infrequently following PHYSO, the rise times of AVP, $ACTH_{1-39}$, and cortisol could not be determined. In the present study, we determined the sequence of hormone increases by frequent blood sampling following PHYSO. Twelve adult women and 12 adult men underwent three test sessions 5–7 days apart: PHYSO, saline control, and repeat PHYSO. As in the earlier study, PHYSO produced no side effects in half the subjects and mild side effects in the other half, with no significant female–male differences. None of the hormone responses was significantly correlated with the presence or absence of side effects. In both women and men, the AVP increase preceded the $ACTH_{1-39}$ increase, which in turn preceded the cortisol increase. The AVP and ACTH AUCs were significantly positively correlated in both women and men, supporting AVP as an acute stimulus to ACTH secretion. Also as in the earlier study, the AVP response to PHYSO was more than twice as great in men as in women, but the difference was not statistically significant. We therefore analyzed the results of both studies combined ($N=26$ women and 26 men). The men had a significantly greater AVP response and a trend toward a greater $ACTH_{1-39}$ response compared to the women. These findings further support the concept of sexual diergism (functional sex difference) in the influence of CNS cholinergic systems on HPA hormone secretion.

Keywords: Cholinergic; Physostigmine; ACTH; Cortisol; Sex difference

Introduction

Cholinergic neurotransmission stimulates both corticotropin releasing hormone (CRH) and arginine vasopressin (AVP; antidiuretic hormone) secretion from the hypothalamus (Gregg, 1985; Tuomisto and Männistö, 1985; Assenmacher et al., 1987; Tsagarakis and Grossman, 1990; Michels et al., 1991; Okuda et al., 1993; Whitnall, 1993; Coiro et al., 1995; Calogero, 1995; Ohmori et al., 1995; Karanth et al., 1999; Rhodes and Rubin, 1999; Rhodes et al., 2001, 2002; Wei et al., 2002). In turn, CRH stimulates ACTH secretion from the anterior pituitary (Rivier et al., 1990). AVP also stimulates ACTH secretion (Antoni, 1993), in addition to promoting water resorption in the kidney.

Cholinergic activation of the hypothalamo–pituitary–adrenal cortical (HPA) axis occurs centrally: Cholinomimetic agents such as physostigmine (PHYSO), a reversible cholinesterase inhibitor that crosses the blood–brain barrier, stimulate the HPA axis, whereas neostigmine, a peripherally acting cholinesterase inhibitor, does not (Janowsky et al., 1986). Similarly, scopolamine, a centrally acting cholinergic receptor antagonist, blocks the effect of PHYSO on the HPA axis, whereas glycopyrrolate, a peripherally acting cholinergic receptor antagonist, does not.

Many previous studies examining cholinergic activation of the HPA axis in humans were confounded by noxious side effects of the administered cholinergic agents, especially nausea

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(Carroll et al., 1980; Davis et al., 1982; Doerr and Berger, 1983, Nurnberger et al., 1983; Lewis et al., 1984; Krieg et al., 1987; Freeman et al., 1990). In contrast, we established a low dose of PHYSO that discernibly elevated plasma AVP, ACTH and cortisol concentrations in normal young adult women and men but produced few or no side effects (Rubin et al., 1999). Prominent findings in that study were 1) significantly greater AVP and ACTH responses to PHYSO in men compared to women, and 2) greater hormone responses in both sexes on the first PHYSO day compared to the second PHYSO day.

Because of the study design, the hormone responses to PHYSO could be calculated on only two 30-min post-PHYSO samples, so that hormone rise times could not be determined. The sample size, as well, was relatively small (14 women and 14 men). We therefore undertook a second study in young adult women and men in which 1) PHYSO again was administered on 2 days, separated by a saline control day; 2) considerably more frequent sampling following PHYSO administration was done, to allow ascertainment of hormone rise times; and 3) no other stimuli were administered following PHYSO, so that complete hormone responses could be calculated. Because the same PHYSO dose was used in these subjects as in the first study (Rubin et al., 1999), to increase sample size, hormone responses also were analyzed in the subjects from both studies combined.

Materials and methods

Subjects

Twelve normal women and 12 normal men matched in age and race were studied. Age- and race-matching were done because of their relationship to HPA axis function (Rubin et al., 1987; Thompson and Rubin, 1993). The subjects were recruited from hospital employees, their families and friends, and through public service announcements. The absence of past or present psychiatric illness in each subject was determined with the Structured Clinical Interview for DSM-IV, Non-Patient Edition (SCID-I/NP) (American Psychiatric Association, 1994; First et al., 1996), conducted by a Ph.D. nurse-clinician. If the subject was suitable for study, the Institutional Review Board-approved experimental protocol was explained in detail, and written informed consent was obtained. The 21-item Hamilton Depression Rating Scale (Hamilton, 1967) also was completed on each subject; Hamilton scores were all ≤ 1.

A physical assessment, complete blood count, urinalysis and urine screen for abuse of common drugs, blood chemistry panel including thyroid indices, electrocardiogram, and chest X-ray (as indicated or if subject was over 40 years of age) then were obtained. Anyone with a history of major medical illness such as hypertension, diabetes, or other endocrinopathy; with abnormal physical or laboratory findings; or who was on any medication that might interfere with the endocrine testing was excluded.

Neuroendocrine protocol

Each subject underwent three test sessions, 5–7 days apart, in the following order: PHYSO, saline control, and repeat PHYSO. The repeat PHYSO day after the saline control day was included because, in our earlier study (Rubin et al., 1999), hormone responses on the first PHYSO day were greater than those on the subsequent PHYSO day. Subjects were blind to the order of drug administration.

For each test session, subjects were admitted to the Allegheny General Hospital Clinical Studies Suite at 1 PM. A heparin-lock catheter was inserted into an arm vein, and 6 mL blood was taken every 30 min starting at 2:30 PM. At 5 PM, a very light meal was given. At 5:30 PM, glycopyrrolate (0.2 mg IM) was given to block the peripheral effects of PHYSO. At 6 PM, PHYSO (8 μg/kg) or an equivalent amount of saline was given intravenously over 2 min. Blood was drawn every 5 min from 6 PM until 6:20 PM, every 10 min until 7 PM, and then every 30 min until 10:00 PM (22 samples; a total of 132 mL).

Each sample was placed into a chilled, silicone-coated glass tube containing 100 μL 15% EDTA in 100 mM phosphate buffer (pH 7.4) and 50 μL 20% sodium azide in 100 mM phosphate buffer (pH 7.4). The tubes were centrifuged for 7–8 min at 500 ×*g*. Aliquots of plasma were immediately pipetted into 1.5 mL conical polypropylene tubes, capped, and frozen at −80 C until analysis for $ACTH_{1-39}$ by immunoradiometric assay (IRMA) and cortisol and AVP by radioimmunoassay (RIA), the details of which have been given earlier (Rubin et al., 1987, 1995, 1999). All samples from each subject were analyzed in the same assay, once for AVP and in duplicate for $ACTH_{1-39}$ and cortisol. As in our first study (Rubin et al., 1999), $ACTH_{1-39}$ and cortisol were measured in all samples, and AVP was measured between 4:30 PM and 7 PM. Hormone values were computed by the four-parameter logistic transformation of the standard curve, followed by dose interpolation of the samples.

Side effects were quantified on a four-point scale (0–3; none, mild, moderate, severe) twice during each session. After the 5:30 PM injection (glycopyrrolate) the criteria were: 0 = none, 1 = metallic taste/mild dry mouth; 2 = prolonged mild dry mouth, 3 = prolonged severe dry mouth. After the 6 PM injection (PHYSO or saline) the criteria were: 0 = none, 1 = transient lightheadedness/nausea, 2 = prolonged lightheadedness/nausea, 3 = vomiting.

Statistical analysis

For $ACTH_{1-39}$ and cortisol, the samples between 2:30 PM and 6 PM served as the baseline afternoon hormone measures. Afternoon baseline cortisol is an estimator of cortisol secretion comparable to the 24-hour average value or the 16-hour post-dexamethasone value (Thompson et al., 1992). For AVP the samples between 4:30 PM and 6 PM served as the baseline measure. Areas under the curve (AUC) were calculated by the trapezoidal rule and used as the measures of hormone response. For $ACTH_{1-39}$ the AUC was 6–9 PM, for cortisol it was 6–10 PM, and for AVP it was 6–7 PM, based on the sampling times and observed response curves for the three hormones (Figs. 1–3). Hormone rise times were determined as the first value >2 SD of the three lowest values prior to PHYSO administration.

Because the women and men were individually matched in age, height, and weight, repeated-measures analyses of variance