deviation and $N^{-1/2}$ has been empirically demonstrated in coupled aggregates of in vitro cultured heart cells, with $1 < N < 100$ [J. R. Clay and R. L. DeHaan, *Biophys. J.* **28**, 377 (1979)]. The model proposed there for pacemaker behavior is quite similar to that in Fig. 1A, but the interpretation proposed for the $N^{-1/2}$ result is derived from empirical data relating amplitude of the noise in membrane voltage with size and number of the heart cells in the aggregate.

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Mood and Behavioral Effects of Physostigmine on Humans Are Accompanied by Elevations in Plasma β-Endorphin and Cortisol

Abstract. *Administration of physostigmine to normal volunteers produced significant elevations in plasma cortisol and β-endorphin immunoreactivity as well as alterations in mood, cognition, and behavior. These observations might be explained by a cholinergically mediated stress syndrome. However, peak elevations in plasma β-endorphin immunoreactivity (but not in plasma cortisol) were significantly correlated with physostigmine-induced increases in depression ratings. These results suggest that a cholinergically mediated β-endorphin pathway may be involved in the observed affective changes.*

The intravenous administration of physostigmine leads to decreases in speech and spontaneous behavior, slowed thoughts, sedation, and occasionally nausea (*1*). Individuals with a past history of affective disorder frequently experience a brief recurrence of depressive symptomatology when given physostigmine (*2*). The drug also has antimanic properties (*3*). Although physostigmine is known to be a cholinesterase inhibitor, with both nicotinic and muscarinic cholinomimetic properties, other aspects of its biological effects on humans have been little explored. Preliminary evidence has suggested that, in some individuals, elevations in cortisol accompany physostigmine administration (*4*, *5*).

Elevations in plasma cortisol levels have been observed in animals and humans under stress and in some psychiatric states, such as depression (*6*). The preliminary reports of elevated plasma cortisol after physostigmine administration raise the possibility that plasma β-endorphin might also be elevated, since cortisol is regulated by adrenocorticotropic hormone (ACTH), and since evidence from animal studies suggests that ACTH and β-endorphin are released together from the anterior pituitary (*7*). Concurrent elevations in ACTH and β-endorphin occur in rats stressed by limb fracture or foot shock; such elevations do not occur in hypophysectomized rats (*7*). Similarly, the synthetic glucocorticoids dexamethasone and prednisolone, which suppress pituitary ACTH release in animals and humans, also suppress, in a dose-dependent manner, the secretion of β-endorphin from normal pituitary gland cultures and from the mouse pituitary cell line AtT-20 (*8*).

We gave physostigmine to normal volunteers in a random-assignment, double-blind crossover study to evaluate whether the behavioral and psychological changes produced by the drug are associated with observable biological (especially neuroendocrine) changes. Nine volunteers, who were screened for the absence of psychiatric illness, were treated with methscopolamine (1.0 mg, intramuscularly) and 20 minutes later with physostigmine (22 μg/kg) or saline intravenously over a 10-minute period. Subjects rated themselves and were rated by a trained observer before and at intervals after the infusions. Blood samples were taken repeatedly for several neuroendocrine measures, including plasma cortisol and β-endorphin immunoreactivity (*9*).

There were significant increases in plasma cortisol ($P < .001$, one-tailed t-test) (Fig. 1) and β-endorphin immunoreactivity ($P < .005$) (Fig. 2) after administration of physostigmine, but not after saline. There was no significant correlation between the increases in plasma β-endorphin and cortisol—surprising, since current evidence supports a common ACTH-β-endorphin pituitary regulatory system. This may reflect recent findings of hypothalamic (*10*) or peripheral (*11*) sources of β-endorphin that are independent of the pituitary regulatory system. Alternatively, since all nine of our subjects had large increases in plasma cortisol while only six had increases in plasma β-endorphin, pituitary ACTH release may be more sensitive to cholinergic stimulation than pituitary β-endorphin release.

In all our subjects, increases in plasma cortisol were 1.9-fold or greater, in contrast to the 17 individuals studied by Davis and Davis (*5*), only six of whom manifested plasma cortisol elevations after intravenous doses of physostigmine (1 to 2 mg). The six also experienced nausea or vomiting, and Davis and Davis (*5*) interpreted the cortisol increase as the sign of a nonspecific stress syndrome. Only three of our subjects developed nausea or vomiting, and there was no significant correlation between self-ratings of nausea or the occurrence of vomiting and changes in plasma cortisol or β-endorphin immunoreactivity.

Our first interpretation of these data, nonetheless, was that the higher frequency of cortisol increases was due to the use of a 10-minute rather than 60-minute physostigmine infusion period, and that the elevations in concentration of the two hormones in the plasma might be re-

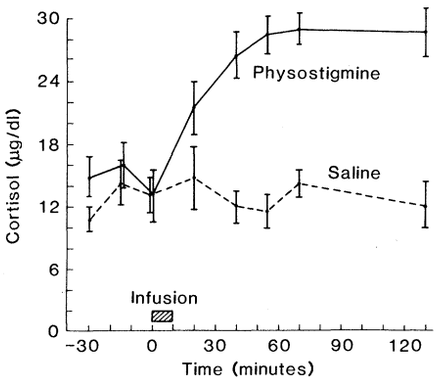

Fig. 1. Changes in plasma cortisol levels (means ± standard errors) after administration of physostigmine or saline in nine subjects. Blood samples were drawn 30, 15, and 0 minutes before the 10-minute infusions and 20, 40, 55, 70, and 130 minutes after beginning them. Paired t-tests were performed to test for significant differences between means.

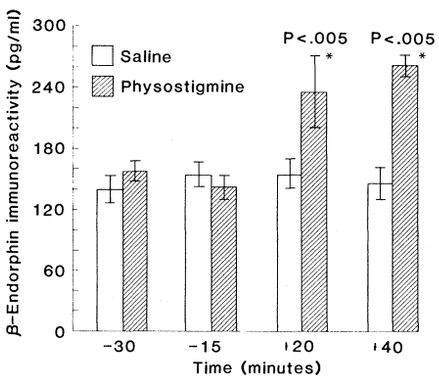

Fig. 2. Changes in plasma β-endorphin immunoreactivity (means ± standard errors) after administration of physostigmine or saline in nine subjects. Blood samples were drawn 30 and 15 minutes before the infusions and 20 and 40 minutes later. Paired t-tests were performed to test for significant differences between means.

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flecting a stress syndrome. As noted above, physical and psychological stress have frequently been associated with cortisol elevations. Similarly, marked elevations in plasma β-endorphin and ACTH concentrations have been reported in mechanically stressed rats (*7*). No comparable data on humans are available, and our observations constitute one of the first reports of a change in plasma β-endorphin immunoreactivity occurring together with a drug-induced alteration in plasma cortisol. Metyrapone, an inhibitor of cortisol synthesis, was previously shown to produce elevations in plasma β-endorphin (*12*).

Another possible explanation for our data was made evident by the psychological and behavioral ratings for our subjects. As expected, a variety of behavioral and mood alterations followed the administration of physostigmine. On both observer rating scales and both self-rating scales, physostigmine led to greater changes than saline for all the scale factors, with many of the scale items yielding statistically significant differences. In general, there were significant affective changes indicative of increased depression and confusion and less vigor and elation—changes closely resembling those observed in other studies of the effects of physostigmine (*1-3, 13*).

Some of the physostigmine-induced psychological changes, particularly in the area of depressive symptomatology, were significantly correlated with the observed elevations in plasma β-endorphin immunoreactivity. These included increases in the depression ($r = .63$), confusion ($r = .66$), and hostility ($r = .65$) subscales of the self-rated profile of mood states scale ($P < .05$) and decreases in the arousal ($r = -.77$) and mania ($r = -.78$) subscales ($P < .05$) and in the total score ($r = -.82$, $P < .01$) of the observer-rated Beigel-Murphy mania scale (*14*). In contrast, no physostigmine-induced changes in behavior or mood were significantly correlated with peak increases in cortisol. We are not aware of any previous report of an association between mood and behavior changes and changes in plasma β-endorphin in humans.

Janowsky *et al.* (*15*) postulated a pharmacologically inducible, cholinergically mediated depressive syndrome. Since the changes we observed in β-endorphin immunoreactivity (but not in cortisol levels or the occurrence of nausea and vomiting) were significantly correlated with the occurrence of depressive symptomatology, a cholinergically mediated β-endorphin pathway may be implicated in the individual variations in affective responses (particularly depression) to a cholinergic stimulus. These results may be of interest in terms of the parallel effects of cholinergic and opiate agonists on such diverse phenomena as motor activity (*16*), learning and memory (*17*), and analgesia (*18*). They also raise the possibility that the cholinergic nervous system has a role in the modulation of endogenous opiate activity. Although the opioid peptides are capable of inhibiting the release of acetylcholine and other neurotransmitters in the peripheral and central nervous systems (*19*), there appear to be no data on whether acetylcholine modulates the apparently discrete β-endorphin–lipotropin system identified in the rat brain, with cell bodies in the arcuate nucleus and long axons innervating midbrain and limbic structures (*20*).

Retrograde transport of pituitary β-endorphin via the hypothalamic portal system, yielding a direct effect on hypothalamic or other brain centers, has been discussed as one possible connection between β-endorphin and brain function (*21*). Further testing of this hypothesis of a linkage between cholinergic and opioid peptide systems capable of altering human affect and behavior will require additional studies—for example, determination of whether naloxone can antagonize physostigmine-induced behavioral and neuroendocrine changes.

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9. Plasma β-endorphin and cortisol determinations were performed with radioimmunoassay kits (New England Nuclear) employing rabbit antiserum to synthetic human β-endorphin and cortisol 21-succinyl bovine albumin, respectively [J. C. Houck, C. Kimball, C. Chang, *Science* **207**, 78 (1980)]. The antibody for β-endorphin has 50 percent cross-reactivity with β-lipotropin but less than 0.1 percent with α-endorphin and α-melanocyte-stimulating hormone and less than 0.004 percent with leucine enkephalin and methionine enkephalin. All blood samples were immediately placed on ice [samples for cortisol determinations in glass heparinized Vacutainer tubes and samples for β-endorphin determinations in polypropylene tubes containing bacitracin (2 mg/ml) and EDTA (20 m*M*) to inhibit proteolysis] and spun at 3000 rev/min in a refrigerated centrifuge. The resultant plasma was immediately placed in a freezer at −80°C until being assayed 1 week to 3 months later. All samples were assayed simultaneously and blindly by the investigators. Statistical evaluation of changes in behavioral ratings, plasma neuroendocrine parameters, and plasma β-endorphin were all performed with one-tailed analyses, since the nature and direction of the physostigmine-induced neuroendocrine (*4*) and behavioral (*1-3*) changes were known from previous studies, and since the direction of the plasma β-endorphin change had been similarly predicted (*7, 8*).
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14. Spearman's rank correlation analysis was used to test for significant relations between behavioral rating changes and changes in levels of plasma β-endorphin immunoreactivity and cortisol. One-tailed tests were used because the directions of the cortisol (*4*), β-endorphin (*7, 8*), and physostigmine behavioral results (*1-3*) were known from previous studies. Pearson product-moment correlations were all significant for these same items in both two-tailed and one-tailed tests, but Spearman's rank analysis was chosen as more conservative for the data.
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