

Assessment of CNS Serotonergic Responsivity
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Serotonin is an important neurotransmitter and peripherally active substance. Interest among mind-body scientists has focused primarily on serotonin as a central/brain neurotransmitter. Psychiatric research has implicated low serotonergic activity in suicide, depression, and antisocial behavior. Subsequent work in behavioral medicine has shown low brain serotonergic activity associated with normal variation in aggressive disposition, hostility, and impulsivity, as well as a number of other important risk factors, such as low socioeconomic status and components of the metabolic syndrome.

Individual differences in central serotonergic function are ordinarily assessed by: (1) lumbar puncture, to evaluate CSF 5-HIAA concentrations; or (2) neuroendocrine responsivity to acutely administered drugs that enhance 5-HT neurotransmission pre- and/or postsynaptically. Although useful as a measure of presynaptic 5-HT metabolism in brain, CSF 5-HIAA reflects, in part, preferential derivation from brain regions proximal to the subarachnoid space and dilution with 5-HIAA from spinal cord itself. Discomfort and risk from lumbar puncture also limits the utility of CSF 5-HIAA measurements in large nonpatient samples.

In the case of neuroendocrine challenges, central 5-HT responsivity is inferred from the relative change (rise) in an "index" hormone following stimulation by pharmacologic agents that act on 5-HT releasing neurons or neurons expressing 5-HT receptors. Such drugs may act through a variety of mechanisms, including potentiation of 5-HT synthesis by tryptophan infusion or administration of 5-hydroxytryptophan (5-HTP), release of 5-HT from storage vesicles and/or inhibition of 5-HT reuptake (e.g., fenfluramine, fluoxetine, citalopram), or direct activation of 5-HT receptors. In the latter regard, although several 5-HT receptor agonists have been employed as neuroendocrine challenges (e.g., m-chlorophenylpiperazine, ipsapirone, buspirone), their use presumes that serotonergic influences on a dependent variable of interest are mediated by one or more known 5-HT receptors. This circumstance does not ordinarily prevail in initial hypothesis testing or where it is postulated (as in the present application) that central 5-HT responsivity may covary with diverse endpoints, both behavioral and biological.

The most widely used neuroendocrine challenge involves administration of fenfluramine hydrochloride, which both stimulates 5-HT release and impedes reuptake by the presynaptic neuron. Stimulation of hypothalamic serotonergic receptors, for example, promotes the pituitary release of PRL into the circulation, even though activity of the neurohypophysis is complexly regulated by several monoamine neurotransmitters (e.g., dopamine). The resulting change in plasma PRL concentration thus provides a relative index of "net" serotonergic responsivity in the hypothalamic-pituitary axis (as believed to be mediated by 5-HT_{2A} and/or _{2C} receptors). This interpretation is supported by dose-dependent PRL responsivity to fenfluramine, by positive correlation between CSF 5-

HIAA and fenfluramine-induced PRL response, and by the inhibition of PRL responses via 5-HT receptor blockade (and, in rats, lesioning of the raphe nuclei). Although PRL changes may also reflect, in part, nonserotonergic influences on the secretory capacity of the lactotroph, PRL response to thyrotropin-releasing hormone has been found unrelated to fenfluramine-induced PRL responsivity.

The specificity of fenfluramine as a 5-HT challenge is not definitive, however, as d,l-fenfluramine may also have dopaminergic and noradrenergic stimulatory effects, and the l- isomer may increase dopamine availability. In any case, fenfluramine is also no longer available as a practicable challenge, due to its withdrawal from manufacture and NIH restrictions on use. As with other investigators, therefore, we have needed to select an alternative challenge, and for the reasons cited above, have again focused on presynaptically acting agents. Candidates include the 5-HT precursors, tryptophan and 5-HTP, and the SSRI, citalopram. Administration of tryptophan promotes 5-HT synthesis and release. From the 1980's until very recently, tryptophan was not commercially available due to product contamination; hence, tryptophan's specificity, reproducibility, and safety remain understudied. When administered acutely, 5-HTP also promotes increased 5-HT synthesis and related neuronal activity. However, conversion of 5-HTP to 5-HT is catalyzed by the relatively ubiquitous aromatic amino acid decarboxylase, thus potentially generating 5-HT at non-serotonergic synapses.

The use of an oral citalopram challenge to assess CNS 5-HT responsivity may be the best current technique to assess central serotonin. Citalopram (Celexa) is an SSRI and therefore differs from fenfluramine in that it is not also a releasing agent. Nonetheless, acute administration of citalopram induces a significant neuroendocrine response (e.g., PRL, cortisol), and this challenge has notable advantages. Citalopram is the most selective and one of the most potent inhibitors of 5-HT reuptake through its transporter system, and it exhibits no known intrinsic activity at 5-HT or other receptor families. Compared to other SSRIs, citalopram produces a more reliable increase in plasma PRL, and both PRL and cortisol responses to citalopram have been found to differentiate depressed individuals and controls. Pharmacologic evidence and extensive clinical experience indicates that citalopram has fewer side effects than other SSRIs, and is safe in diverse populations. While it might be preferable to administer the drug intravenously, the parenteral formulation has not been approved for use in the U.S. and is not available through the U.S. distributing firm, Forest Pharmaceuticals; in addition, oral citalopram has high bioavailability ($\geq 80\%$).

Dr. Kurt Ackerman at our institution found that 40 mg of orally administered citalopram induced a mean PRL rise of 3-3.5 ng/ml over 5 hr in 35 euthymic women (mean age: 37; weight: 69 kg). In our own work we selected a dose of 0.9 mg/kg lean body mass provides the same mean dose that Dr. Ackerman administered. Among 16 subjects studied, the peak PRL response averaged 3.65 ng/ml (i.e., equivalent to fenfluramine under our modified protocol) and varied appreciably among individuals. Side effects (nausea, dizziness, headache) were occasional and tolerable. As importantly, we found a retest reliability (evaluated in 12 subjects) of .66 ($p = .02$), which is also comparable to our experience with fenfluramine (see Progress Report). More recently, we examined

the predictability of the PRL response to citalopram from corresponding PRL change induced by fenfluramine ($r = .51$, $p = .02$). This association is notable for the length of interval separating administration of the fenfluramine and citalopram challenges, which averaged 4.5 years. In addition, with Dr. Jay Kaplan from the Bowman Gray School of Medicine, we compared PRL responses evoked by both fenfluramine and citalopram in 20 cynomolgus monkeys; fenfluramine dose was 4 mg/kg IM and citalopram, 3 mg/kg IM. Two timed blood samples were obtained over 1 hr, and unprovoked PRL levels were established by sham injection on an alternate day. The two challenges were administered one month apart. Rank order (Spearman) correlation of PRL responses to the two challenges was 0.52 ($p < .02$) and nominal classification of animals as “high” or “low” PRL responders by median division of the response distribution on each challenge yielded concordant classification in 80% of animals (i.e., 16 of 20; $p < .05$).

Procedure: Subjects are typically studied in the morning after an overnight fast. Our protocol includes insertion of an intravenous catheter, 30-minute adaptation period, citalopram administration, and sequential blood sampling for PRL and ACTH. Samples are obtained every 60 minutes for 5 hours. Citalopram levels are also determined from blood sampled at 4 hours, the approximate time of peak drug concentration. Blood samples should be placed immediately in an ice bath and centrifuged at 4° C to separate plasma. All samples can then be stored at -70° C until assayed. Subjects remain fasting throughout the challenge.