Neuropsychopharmacologic Challenge in Biological Psychiatry

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Psychopharmacologic challenge procedures offer many useful features for biological psychiatry research. First, challenge procedures acutely stimulate neurotransmitter and (or) hormonal systems in the brain and offer the chance to assess the function, and (or) reserve, of the brain neurotransmitter/hormone system of relevance. Second, these paradigms often offer functional information about specific areas of the brain (e.g., limbic-hypothalamus). Regardless of these advantages, the power of these procedures is limited to the specificity of the agent and of the functional anatomy of the "circuit" that leads to the outcome measure, which, in turn, is linked to the stimulation by the acute pharmacologic challenge. We describe here the development of the psychopharmacologic challenge methodology and the strengths and weaknesses of this approach. Finally, the application of this paradigm in the study of various neuropsychiatric disorders will be reviewed and critiqued.

Indexing Terms: mood disorders/neurotransmitters/receptor agonists/serotonin/prolactin/pituitary hormones

One substantial component subsumed by the field of biological psychiatry involves the study of bodily substances with relevance to central nervous system (CNS) function. These substances fall into several categories and include neurotransmitters (including metabolites and precursors of neurotransmitters), cellular receptors or other structures, and hormones. Historically, this branch of biological psychiatry focused simply on the measurement of these substances to determine whether there were any differences in their concentrations in patients with psychiatric disorders and in healthy volunteers. If differences were found, they were interpreted as evidence that the brain system related to the substance in question was different in the psychiatric patients studied.

Bodily substances available for study in this way have been obtained from a variety of sources, most commonly urine, blood, and cerebrospinal fluid (CSF) (Table 1). Not surprisingly, there is an inverse relation between the ease of obtaining samples from these sites and of their proximity to the CNS. Although urine collection is one of the least invasive methods known for collecting bodily fluids, it is the least informative source for many brain systems [e.g., serotonin (5-HT), dopamine]. Moreover, it has become increasingly difficult, if not unfeasible, to collect the required number [usually three (1)] of complete 24-h samples in most subjects in most research settings.

Measurement of receptor elements on peripheral blood cells offers the trade-off of a more "invasive" procedure with the advantage of assessing cellular structures that may closely parallel those on CNS neurons (2). At least one caveat, however, obtains: Because these receptor elements are exposed to the peripheral circulation, they exist in an environment quite different from that of their counterpart structures in the CNS, which are under the direct influence of physiological changes in neurotransmission at the synaptic level. Hence, studies of peripheral blood elements may not always reflect the influences of pathology in the CNS. Moreover, studies of blood samples cannot identify abnormalities that might be localized to one brain region or another.

Measurement of substances in the CSF requires a lumbar puncture, one of the most invasive collection methods currently used in biological psychiatry. On the other hand, analysis of CSF offers an examination of the concentrations of various substances that actually come into contact with the substance of the brain. Accordingly, there is a greater probability that substances measured in the CSF reflect the concentrations of substances produced in the course of brain activity, or, at least, produced close enough to brain cells to affect their function (3). Despite these advantages, CSF measures cannot reflect differences in neuronal activity in specific brain regions. In addition, small differences in regional concentrations of various substances may not be detected because of the size of the CSF compartment. Hence, more subtle, though potentially important, regional differences in brain function (e.g., as reflected by neurotransmitter release) may not be identified through this method.

The Psychopharmacologic Challenge

By the mid-1970s, biological psychiatrists began to develop a new methodology designed to enable a dynamic assessment of brain function: the psychopharmacologic challenge. A small dose of a psychoactive agent is given, and any of a variety of behavioral or physiological responses are monitored over time. Accordingly, the magnitude of these responses is interpreted as reflecting the functional integrity of a specific synaptic pathway or the sensitivity of a neuronal receptor. Here, we limit our discussion of the various properties and characteristics of the psychopharmacologic challenge method to those agents directed at specific neurotransmitter systems. Challenge formats that produce behav-

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1 Author for correspondence. Fax 215-842-4321.
2 Nonstandard abbreviations: CNS, central nervous system; CSF, cerebrospinal fluid; AUC, area under the curve; 5-HT, serotonin; m-CPP, m-chlorophenylpiperazine; MHPG, methyldihydroxyphenylglycol; and PRL, prolactin.

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Table 1. Advantages and disadvantages of biological measures from urine, blood, and CSF.

<table>
<thead>
<tr>
<th>Type</th>
<th>Advantages</th>
<th>Disadvantages</th>
</tr>
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<tbody>
<tr>
<td>Urine</td>
<td>Metabolites, hormones</td>
<td>Cumbersome, peripheral, no localization</td>
</tr>
<tr>
<td>Blood</td>
<td>Plasma, platelets, lymphocytes</td>
<td>Peripheral, no localization</td>
</tr>
<tr>
<td>CSF</td>
<td>Metabolites, hormones</td>
<td>Minimal localization</td>
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ioral responses through unclear, possibly noncentral, mechanisms (e.g., lactate infusion-associated panic attacks) are not included.

Anatomy. The underlying principle of the psychopharmacologic challenge is that the data yielded reflect the responsiveness of the neuronal system being challenged. For example, if the challenge agent stimulates postsynaptic noradrenergic receptors, the magnitude of the measured physiological response (e.g., growth hormone release) reflects the functional sensitivity of these receptors; moreover, the receptors in question are located in a specific region on the brain (e.g., hypothalamus). The region of the brain affected by the challenge agent depends on the type of outcome measure being examined. For example, anterior pituitary hormonal responses (e.g., prolactin (PRL), growth hormone, corticotropin) may be localized to activity at the level of the hypothalamic-pituitary axis. Thermal responses may also be localized to the hypothalamus. One potential advantage in using thermal responses to challenge agents (i.e., where this can be utilized) is that abnormal responses cannot be due to abnormalities further distal to the hypothalamus, as might be the case where anterior pituitary hormonal responses are used. Behavioral responses to challenge agents are likely to be of extrahypothalamic origin. In addition, these offer the advantage of stimulating behaviors of interest rather than of simply a hormonal or thermal response, where the relevance of the latter responses must be inferred (Table 2).

Dose-response nature of the paradigm. A critical characteristic of the psychopharmacologic challenge is that the outcome (e.g., anterior pituitary hormonal response, thermal response, or behavioral response) should demonstrate a dose-response relationship with the challenge agent. One of the critical assumptions of this paradigm is that the outcome marker has the capacity to reflect differences in physiological responsiveness to challenge. This is especially important where only one dose of the challenge agent is given to patients and controls. It would be ideal to compare the outcome measure of interest in patients and controls receiving several different doses, but this is nearly impossible to do in clinical biological psychiatric research, given the limits in terms of subject tolerance to repeated studies and the need to move as quickly as possible to active treatment. Hence, it is critical that initial studies with specific challenge agents include sufficient determination of dose-response, at least with healthy volunteers.

Given the difficulty in performing dose-response studies in patient/control comparison studies, the hypothesis to be tested must be focused as narrowly as possible. For instance, is the hypothesis predicting that the patients in question will have higher (or lower) responses than the control? If the expectation is that patients will have a higher response than controls, the investigator may be advised to choose a challenge dose at the low end of the dose-response curve, so that a “hypersensitive” response in patients may be detected. If the dose of the challenge agent is too high, then all subjects will respond, which will diminish any hypothesized differences between patients and controls. The opposite case is more difficult to predict. In cases where the hypothesis predicts that patients will have a lower response than controls to the challenge agent, one must consider whether it is better to choose a dose at the top or at the middle of the dose-response curve. Doses at the top of the curve could produce essentially equivalent responses among patients and controls, even when there might be differential responsiveness at lower doses. This is important because of the possibility, often probability, that doses at the top of the dose-response curve will reflect activation at supraphysiological amounts of the agonist and therefore not reflect synaptic/receptor responsiveness at lower, more physiological amounts of neurotransmitter, i.e., those that occur at impulse-driven neurotransmission. Accordingly, selecting a dose somewhere in the middle of the dose-response curve, where possible, is usually more informative. In such cases, small differences in synaptic/receptor sensitivity may be better detected because the challenge is taking place in the steeper part of the curve.

Assessment of response parameter. The pharmacological challenge outcome variable can be expressed in a variety of ways, including the absolute magnitude of the variables after the challenge; the peak value minus baseline value (i.e., “peak delta”); the area under the curve (AUC); and placebo-corrected delta or AUC. Statistical analysis by analysis of variance with repeated
measures is usually performed on the values obtained at all time points. However, when only one response marker is expressed, or is preferable (e.g., for correlations with other variables), the peak delta and AUC values are most commonly used. Thus, it is good practice to demonstrate that the peak delta correlates with the AUC, even in cases where the peak delta more accurately reflects the effect of the pharmacochallenge on the outcome variables—a e.g., when the challenge drug takes a substantial time to reach effective plasma concentrations. In such cases, outcome variables obtained before the time of effectiveness will reflect nonspecific factors rather than the effect of the pharmacochallenge agent itself. “Placebo-correction” of peak delta or AUC values is useful where the outcome markers are temporarily unstable (e.g., corticotropin or cortisol through the day). By subtracting the variability of outcome variables attributable to nonspecific/nondrug factors, placebo-correction offers a response measure that is due to the effect of the drug alone.

Effect of baseline physiology on responsiveness. The influence of basal values of the outcome marker is often critical to the interpretation of pharmacochallenge data. The basal value of the outcome marker is often correlated with the response of the marker to the challenge agent. Sometimes the apparent effect can be “corrected,” depending on how the outcome marker is expressed in the data analysis. For example, if the analysis examines only the absolute values of the outcome marker after the challenge, using a delta score (i.e., subtracting the baseline value) may eliminate the effect of different basal values of the marker among subjects. Another option is to analyze the postchallenge values as a function of the basal values of the outcome marker. If the basal values simply increase the outcome values of only one group, responses should be analyzed as a percent of baseline to address this problem. More usually, however, basal values appear to actually influence the value of the response marker postchallenge. In such cases, there will be a correlation between the basal value and the measure of “responsiveness” to the challenge probe (4). The direction of the correlation may have physiological significance. For example, in examining PRL response to agonist challenges, a positive relationship between basal PRL and delta PRL could suggest that basal dopamine tone at the level of the pituitary (5) has a direct relationship with PRL responsiveness to the agent in question. The lower the dopamine tone, the less inhibition there may be at the pituitary lactotrophs, and the greater the response to the challenge agent (6). This possibility is important because it would suggest that part of the PRL response to the agent in question reflects activity at the level of the pituitary. Because biological psychiatry is generally interested in activity above the level of the pituitary, it is important to rule out or confirm this possibility in further studies (e.g., by comparing the PRL response with the response to thyrotropin-releasing hormone, an agent that stimulates PRL only at the pituitary level (7)).

Effects of challenge agent pharmacology. The pharmacological properties of the challenge agent are, of course, essential to the utility of the paradigm. For orally administered agents, such properties relate to the absorption, first-pass metabolism, and distribution of the agent under study. In turn, each of these contributes greatly to the concentration of the agent in the plasma. (The plasma concentration is only an approximation of the concentration of challenge agent that may be available at relevant synaptic sites, but it is the only feasible way to assess the pharmacological availability of the agent in question.) Accordingly, an appropriate pharmacochallenge agent should be well absorbed after oral administration, have minimal first-pass metabolism through the liver, and be distributed widely in the tissues of the brain.

These last two issues are important for several reasons. The effects of first-pass metabolism, for example, have multiple ramifications. First, this process may significantly diminish the amount of the agent available for systemic absorption. Second, the plasma concentrations of the agent may vary widely within a sample of subjects because of great interindividual differences in the rate at which these compounds are metabolized. Because this effect is due to genetic variability in hepatic metabolism, attempts to standardize dosing according to weight are unlikely to correct for it. Third, the agent may be metabolized to products that themselves have confounding pharmacodynamic effects. A case in point is the arytpiperazines (e.g., buspirone), which are extensively metabolized to 1-phenylpiperazine, an agent with a2-noradrenergic-blocking properties (8). For those agents for which the 5-HT1a agonistic properties are of greatest interest, the presence of a2 antagonism is clearly an undesirable confounding variable.

Finally, the ability of an agent to penetrate the blood–brain barrier, a property determined by the lipophilic nature of the compound, is most critical in determining whether the agent will actually be available at the relevant synaptic sites. Unfortunately, some highly selective and interesting agents currently available for human study are not very lipophilic and cannot make useful pharmacochallenge agents, e.g., sumatriptin, a selective 5-HT1d receptor agonist.

Pre- vs postsynaptic receptors. Regardless of the outcome marker selected, there is always the question about what part of the synaptic system is being stimulated. The first clue is given by the mechanism of action of the challenge agent. If the challenge agent is a neurotransmitter precursor, as in the case of L-tryptophan, the primary action of the agent is within the presynaptic terminal (9). The assumption is that L-tryptophan is taken up into the presynaptic 5-HT terminal, converted into 5-HT, packaged into synaptic vesicles, and then released on nerve impulse (10). This is consistent with the fact that newly synthesized, rather than stored, 5-HT is preferentially released on nerve impulse. Accordingly, physiologic (e.g., hormonal) responses to challenge with L-tryptophan appear to reflect activity at the presynaptic side of the 5-HT synapse. However, this
conclusion is only partly true: Preclinical studies demonstrate that hormonal responses associated with stimulation of 5-HT pathways are a 5-HT-postsynaptic-mediated effect (11–14). Removal of the postsynaptic 5-HT input may diminish, or abolish, the hormonal response to 5-HT stimulation, but only after acute lesioning (13, 15). Follow-up after chronic 5-HT depletion reveals that 5-HT stimulant challenge (with direct postsynaptic 5-HT agonists) produces an extremely robust hormonal response (12). Moreover, hormonal responses to challenge with t-tryptophan are blocked by nonselective postsynaptic 5-HT receptor antagonists (16). Hence, the hormonal response to t-tryptophan challenge depends on both preand postsynaptic factors. Postsynaptic factors include the functional integrity of the tryptophan uptake site, the metabolic apparatus converting tryptophan to 5-HT, the cellular apparatus that stores and releases the 5-HT upon nerve impulse, and the functional integrity of the 5-HT uptake site (which inactivates synaptic 5-HT). Postsynaptic factors include the functional responsivity of the postsynaptic receptors and second messenger system (which propagates the signal distal to the synapse) and the intermediary steps that lead to the production of the outcome marker (e.g., hormonal response). The same discussion is generally true for other indirect agonist-type agents, which either: (a) release neurotransmitter stores (e.g., fenfluramine), (b) block synaptic uptake (e.g., desipramine, clomipramine), or (c) inhibit catabolic enzymes that otherwise deactivate the neurotransmitter in the synapse (e.g., physostigmine). In these cases, however, the postsynaptic receptor complex plays an increasingly larger role in determining the magnitude of the outcome marker. For releasing and uptake-inhibiting agents, the role of precursor uptake sites (if applicable) and the use of synthetic enzymes are irrelevant to the outcome marker because these agents completely bypass these presynaptic mechanisms. Obviously, none of the above mechanisms is relevant when a direct-acting postsynaptic receptor agonist [e.g., clonidine, m-chlorophenylpiperazine (m-CPP), ipsapirone] is used, because these agents completely bypass presynaptic mechanisms in their actions. This does not, however, rule out indirect effects of presynaptic factors: Chronically diminished presynaptic output might lead to supersensitive postsynaptic receptors, which could be manifest as enhanced responsiveness to the agonist (17, 18).

Although we can assume that receptor agonists simply stimulate postsynaptic receptors, in many cases these agents also have effects on presynaptic autoreceptors. The α2 receptor agonist, clonidine, stimulates both pre- and postsynaptic α2 receptors. Stimulation of the presynaptic receptors is associated with a decrease in plasma concentrations of norepinephrine and its major metabolite methylhydroxyphenylglycol (MHPG) (19), whereas stimulation of the postsynaptic receptors is associated with an increase in growth hormone release (20). The effect of the α2 receptor antagonist, yohimbine, on presynaptic receptors is the reverse of that seen with clonidine (21) because blockade of the presynaptic α2 autoreceptor leads to an increase in norepinephrine release and consequently an increase of plasma MHPG.

Limits of resolution regarding receptor-specific subtypes. Despite claims that certain probes are selective for one receptor subtype or another, most available probes are perhaps only relatively selective. Agents that are only indirect agonists are, by definition, the least selective in terms of receptor subtype specificity. Such agents enhance the activity of the endogenous neurotransmitter, which then crosses the synaptic cleft and acts as it would under physiological conditions. Specificity for receptor subtype could exist with these probes only if the outcome marker itself is receptor-subtype specific. At present, however, this does not seem to be the case for most of these agents and their outcome markers. Agents that are direct agonists may also be hindered because they, by virtue of their molecular configuration, bind potently to more than one receptor subtype for any neurotransmitter. For example, m-CPP can bind to many of the 5-HT receptor subtypes, e.g., 5-HT-1a, 5-HT-1c, 5-HT-1d, 5-HT-2, 5-HT-3 (22). An important issue here is, does m-CPP act as an agonist or as an antagonist at these receptor subtypes? Preclinical data suggest that m-CPP acts as an agonist at 5-HT-1a and 5-HT-1c receptors and as an antagonist at 5-HT-2 receptors (23, 24). As long as the outcome markers are not affected by 5-HT-2 antagonism, then m-CPP could reflect receptor responsivity at the 5-HT-1a and 5-HT-1c sites. However, because m-CPP is far more potent in its binding affinity for the 5-HT-1c site than for the 5-HT-1a site (25), its physiological outcome marker will probably reflect mostly the 1c receptor site.

Stability of response over time. Another important characteristic critical to the utility of any biological marker of outcome is stability over time when all other conditions are the same. In studies of psychiatric disorders in which changes in clinical state are common and clinically important (e.g., mood disorders, schizophrenia), knowledge of the temporal stability of the marker is obviously relevant. If the underlying abnormality characterizing a particular neurotransmitter system does not change between the ill state and the well state but the biological marker is inherently unstable (and conversely), erroneous assumptions will be made about the neuron system under study. Unfortunately, little is known about the temporal stability of many pharmacological markers. Such agents exhibit some short-term test-retest reliability, but only for a few subjects per challenge agent (26–29, Trestman et al., ms. in review).

Equally important is the effect of repeated challenges over a relatively short time. Some investigations use repeat pharmacological challenge testing to determine whether treatment with various psychotropic agents affects the specific neurotransmitter systems targeted by the challenge agent. In such studies, it is important to know that rechallenge of similar patients undergoing placebo treatment will demonstrate a significant difference in responsiveness from those rechallenged during short-term treatment. Unfortunately, this methodological feature is absent from nearly all published studies that
rechallenge subjects after treatment with a psychotropic agent.

Short-term stability is also important in receptor antagonist studies in which the pharmacochallenge is repeated in the presence and absence of a specific receptor antagonist. The objective is to demonstrate whether a specific receptor mediates the biological or behavioral response in question. If the initial challenge affects subsequent responses to repeat challenges, the data will obviously be confounded. One way investigators attempt to control for this potential problem is to randomize the sequence of challenges. The optimal method, however, is to determine the appropriate interval between pharmacochallenges and to design the study to use these intervals. This issue becomes even more important for dose–response studies with pharmacochallenge agents, especially when the dose of the agonist challenge is kept constant between challenges and the dose of the receptor antagonist is gradually increased over the course of the study. In this case, the potential for a progressive diminution in response with repeat challenge can interact with the potential for the receptor antagonist to blunt the response because of its own pharmacological properties.

This issue is of more than academic concern: Biological or behavioral responses to pharmacochallenge could diminish over repeated dosing, even for dosing as infrequent as once a week. Thyrotropin responses to thyrotropin-releasing hormone stimulation decreased progressively over four challenges repeated weekly (30). In addition, PRL responses to intravenous clomipramine may be diminished for as long as 2 weeks after one challenge. The same may be true of D,L-fenfluramine in some adults (27), although the most recent study of this showed no evidence of short-term reduction in PRL responsiveness in young children (28). This seems also to be true for growth hormone responses to intravenously administered clonidine in healthy volunteers for whom challenges were separated by 24 h or by weeks (Trestman et al., ms. in review). Curiously, however, PRL responses to the direct 5-HT agonist m-CPP may be affected by prior dosing only 24 h before (Coccaro et al., unpublished).

Other confounding factors in interpretation of data. Factors seemingly unrelated to the specific pharmacology of the pharmacochallenge agent often play a factor in the interpretation of data obtained in pharmacochallenge studies. These factors include age, weight (adiposity), recent weight loss, menstrual status (including time of menstrual cycle), and influence of pretreatment with other agents.

Age may influence the interpretation of pharmacochallenge data, and the effect of age should always be investigated. In general, hormonal responses to pharmacochallenge are diminished by age (23), although the magnitude of the effect is often small. Age-matching of subjects can do much to diminish the effect of age on the outcome variables, although analysis of covariance is usually needed as well. The effect of age on outcome variables is probably due to a decrease in the number and (or) sensitivity of relevant postsynaptic receptors with age (31).

Body weight may also influence the results. Like age, body weight tends to be inversely related to pharmacochallenge outcome markers. Where the challenge dose is fixed, this effect may be due, to some degree, to an inverse relation between body weight and the plasma concentration of the challenge agent. However, body weight may still influence pharmacochallenge variables, even when the dosing scheme corrects for variability in body weight. Hence, like age, a relationship with body weight should always be investigated in any pharmacochallenge data set. In addition, increasing degrees of adiposity may diminish the pituitary response to pharmacochallenge agents, particularly where the outcome marker is growth hormone and when the subject is frankly obese (32). Finally, there is the potential effect of recent weight loss. Recent weight loss, specifically in females, is reported to increase PRL responses to L-tryptophan challenge (33). Because it is sometimes difficult to weight-match subjects, statistical correction (analysis of covariance) is usually used when this effect is present.

Menstrual status also has a powerful effect on pharmacochallenge outcome markers, particularly hormonal variables. The arrival of menopause is a milestone that is usually associated with a diminution of hormonal responsiveness to pharmacochallenge. Hence, women who are premenopausal should not be included in the same analysis as postmenopausal women. In fact, premenopausal women should not be included in an analysis of men of similar ages unless the potential effect of gender is investigated in the specific data set. This is because many studies demonstrate a significantly greater (e.g.) hormonal response to pharmacochallenge for women than for men of a similar age (31, 34). In addition, evidence is emerging that time of menstrual cycle affects hormonal responses to some challenge agents, e.g., buspirone (35) and d-fenfluramine (36). In general, it is becoming standard practice to conduct challenge studies no later than 7–10 days after the onset of the last menstrual cycle. Although not fully understood, rising concentrations of estrogenic hormones appear to sensitize the receptor responsiveness of at least some neurotransmitter systems (37, 38).

Another factor that can affect the interpretation of pharmacochallenge data is the aftereffect of previous drug treatment. Little is known about how great a time period should elapse before investigation; however, it is standard practice to allow at least a 2-week (if not greater) drug-free period to elapse before administering a pharmacochallenge. Regardless, a possible relationship with the drug-free interval should always be investigated and, if found, should be corrected statistically. This is because pharmacochallenge studies, performed on patients undergoing treatment with various psychoactive drugs, indicate direct effects of these agents on various pharmacochallenge outcome markers. Specifically, treatment with cyclic antidepressants tends to decrease the physiological responses to clonidine chal-
lenge (17)—which is consistent with their effects to diminish noradrenergic receptor sensitivity (39). However, if a depressed patient, for example, were studied too soon after treatment with a cyclic antidepressant, it would be unclear whether the reduced physiological responses to clonidine challenge were primarily associated with the depressive disorder or with the recent treatment with a cyclic antidepressant. This could lead to a type I error. Conversely, treatment with cyclic antidepressants and 5-HT uptake inhibitors tend to be associated with enhanced hormonal responses to 5-HT agent challenge (21, 40, 41). If treatment of a depressed patient were too close to a challenge with these agents, a type II error could result: i.e., the hypothesized blunted hormonal response to 5-HT challenge could be masked by an enduring pharmacodynamic effect on the 5-HT system.

Characteristics of Commonly Used Psychopharmacologic Challenges
Catecholaminergic Agents

Mixed. The first pharmacochallenge agents to be utilized in biological psychiatry were mixed catecholaminergic agents (17), including the amnergic-releasing d-amphetamine and the catecholaminergic precursor L-dopa. Of the two, d-amphetamine is the more nonselective pharmacochallenge agent. Depending on dose, d-amphetamine will activate noradrenergic, dopaminergic, and serotonergic systems. Although this degree of nonselectivity is ordinarily a disadvantage, d-amphetamine, unlike more select agents, produces a rich variety of behavioral responses, including excitation, euphoria, dysphoria, and perceptual and cognitive distortions, depending on the individual (42–44). Pharmacological dissection of these responses has yet to be completed, but preliminary data suggest that the "excitation" response may reflect stimulation of the dopaminergic system (42). Preliminary data also suggest that the growth hormone response to d-amphetamine correlates with increases in ratings of thought disorder in patients with borderline personality disorder (44). As a precursor for both dopamine and norepinephrine, challenge with this agent activates both catecholaminergic systems (17). L-dopa/carbidopa (Sinemet®) challenge suppresses PRL and stimulates growth hormone. Suppression of PRL secretion is thought to reflect suppression of pituitary lactotroph activity through enhancing the tuberoinfundibular dopamine function. Stimulation of growth hormone secretion, however, may reflect stimulation of pituitary somatotrophs through both enhancement of dopaminergic and noradrenergic systems.

Dopamine selective. The two most common dopaminergic pharmacochallenge agents are apomorphine and bromocriptine. Both are dopamine-2 receptor agonists and, as such, stimulate growth hormone and suppress PRL secretion, respectively. Apomorphine challenge requires intramuscular administration; bromocriptine may be given orally. To date, there has been far more research with apomorphine (45) as a challenge probe than with bromocriptine.

Noradrenergic selective. The most common noradrenergic challenge agents include the selective norepinephrine uptake inhibitor desipramine, the α2-adrenergic receptor agonist clonidine, and the α2 receptor antagonist yohimbine. Desipramine is administered either intravenously or intramuscularly (routes that avoid first-pass hepatic metabolism); administration by either route stimulates the secretion of both growth hormone and cortisol. The attenuation of the growth hormone response by phenotolamine (an α1 and α2 receptor antagonist) and yohimbine (an α2 receptor antagonist) but not by propranolol (a β1 and β2 receptor antagonist) or by prazosin (an α1 receptor antagonist) suggests that this response is mediated by α2 postsynaptic receptors (46). Attenuation of the cortisol response by prazosin, and enhancement by yohimbine, suggest that this response is mediated by both α1 (stimulatory) and α2 (inhibitory) postsynaptic noradrenergic receptors (47).

Both clonidine and yohimbine are more selective than desipramine as α2 challenge agents. Clonidine challenge stimulates growth hormone secretion and inhibits (or has no effect on) cortisol, plasma norepinephrine, and plasma MHPG. The growth hormone and cortisol responses are thought to be mediated by stimulation of postsynaptic α2-noradrenergic receptors (20), whereas the plasma norepinephrine and MHPG responses are thought to be mediated by stimulation of presynaptic α2-noradrenergic autoreceptors (19). Clonidine can be administered by either the intravenous or oral route. As an α2-noradrenergic antagonist, yohimbine's profile is generally the opposite of clonidine. Yohimbine challenge stimulates plasma MHPG release by blockade of presynaptic α2-noradrenergic autoreceptors (48). Besides increasing plasma MHPG, yohimbine also increases the levels of subjective anxiety, particularly in patients with anxiety disorders such as panic disorder (21). In contrast, clonidine challenge is associated with a small but statistically significant reduction of subjective anxiety levels (49).

Serotonergic Agents

Indirect agonists. This group of serotonergic agents includes the 5-HT precursors L-tryptophan and 5-hydroxytryptophan, the 5-HT-releasing/uptake-inhibiting agent d,l-fenfluramine and d-fenfluramine, and the 5-HT-uptake-inhibiting agent clomipramine. Of the 5-HT precursor pharmacochallenge agents, L-tryptophan has been the most well studied. L-Tryptophan is given intravenously and produces reliable increases in the secretion of PRL and growth hormone (9, 50, 51). The PRL response is attenuated by the nonselective 5-HT receptor antagonist metergoline (16) and enhanced by the 5-HT-1c and 5-HT-2 receptor antagonist ritanserin (52). The PRL response also is partially attenuated by the 5-HT-1a receptor antagonist pindolol (53) but not by a 5-HT-3 receptor antagonist (54). This suggests that the PRL response to L-tryptophan may be mediated by 5-HT-1a (stimulatory) and 5-HT-1c/2 (inhibitory) postsynaptic 5-HT receptors. The receptor dissection of the growth hormone response is more contro-
versial. Metergoline pretreatment has no effect on the growth hormone response (16), but pindolol pretreatment almost fully blocks this response (53). This suggests, pending further data, that growth hormone response to L-tryptophan may be mediated by 5-HT-1a postsynaptic receptor activity.

Challenge with 5-hydroxytryptophan, which, unlike L-tryptophan, is administered orally, leads to a reliable increase in cortisol secretion. In animal studies, this response can be attenuated by pretreatment with nonselective 5-HT receptor antagonists (55). Similar studies in humans suggest that this response can be attenuated by acute pretreatment with ritanserin (56). Hence, cortisol responses to 5-hydroxytryptophan challenge may reflect activation at 5-HT-1c or 5-HT-2 receptors, or both. Further study with other 5-HT receptor antagonists such as pindolol (5-HT-1a) and ondansetron (5-HT-3) will be necessary before the receptor subtyping of these hormonal responses can be fully understood.

Both d,l-/d-fenfluramine and clomipramine challenge lead to reliable increases in PRL secretion (57–59). d,l-Fenfluramine and d-fenfluramine differ in that the former is a racemic mixture of the d- and l-stereoisomers, whereas the latter is a pure preparation of the d-stereoisomer. Preclinical data suggest that the l-stereoisomer has potent antidopaminergic effects that are lacking in the d-isomer (60). Whether this difference is in fact clinically important is yet to be determined. PRL responses to both agents may be attenuated by nonselective 5-HT receptor antagonists. Little is known, however, regarding the receptor subtyping for this response, although animal studies suggest that the PRL response to d-fenfluramine is mediated by 5-HT-1a (61) and (or) 5-HT-1c/2 (62) postsynaptic 5-HT receptors.

Direct agonists. This group includes the postsynaptic receptor agonists m-CPP, buspirone, and ipsapirone. m-CPP challenge leads to reliable increases in secretion of PRL, corticotropin, and cortisol (but not growth hormone) (63, 64). m-CPP can be given either orally or intravenously. Despite early reports that it was selective for the 5-HT-1 receptor subtype, m-CPP also binds to other 5-HT receptors (25). Accordingly, m-CPP represents a nonselective postsynaptic receptor agonist. Attenuation of these hormonal responses to the nonselective receptor antagonist metergoline (64) and partial attenuation by the 5-HT-1c/2 receptor antagonist ritanserin (GR Heninger et al., unpublished), but not by the 5-HT-3 receptor antagonist ondansetron (65), suggests that these hormonal responses are mediated by 5-HT-1c or 5-HT-1a receptors (m-CPP is an antagonist at 5-HT-2 receptors; see above).

Buspirone, a partial 5-HT-1a agonist, has been utilized as a putative 5-HT-1a receptor probe in various challenge studies in humans. Unfortunately, buspirone has significant inhibitory effects on dopaminergic systems (66), which limits its attractiveness as a selective 5-HT probe (67). Unlike buspirone, however, ipsapirone is a fuller agonist at 5-HT-1a receptors and has minimal effects on the dopaminergic system in animal studies (68). Although its administration is associated with metabolic conversion to 1-phenylpiperazine, empiric studies indicate that very little of this metabolite is produced at the doses administered during a single-dose ipsapirone challenge (69). Ipsapirone produces reliable increases in secretion of corticotropin and cortisol (69). These signals are considered postsynaptic in nature and can be blocked by the nonselective 5-HT receptor antagonist metergoline and the more selective 5-HT-1a receptor antagonist pindolol (69). The fact that these responses cannot be blocked by the selective β2 antagonist betaxanol suggests that the pindolol blockade represents the effect of 5-HT-1a receptor blockade. Ipsapirone also produces a decrease in body temperature, an effect interpreted as a presynaptic 5-HT-1a effect (70).

In conclusion, the neuropsychopharmacologic challenge constitutes an important and powerful strategy with which to examine the physiological responsiveness of brain neurotransmitter systems in clinical samples. While having several advantages over older methods, the neuropsychopharmacologic challenge has some clear limitations (Tables 1 and 2). For example, localization of abnormal function to a particular brain region is limited by the nature of the outcome variable (e.g., limbic—hypothalamus for hormonal outcome variables). In addition, the specific pharmacologic properties of the agent chosen for study can limit the interpretive power of the paradigm (e.g., lack of specificity for neurotransmitter system or receptor subtype). However, if these and other methodological factors (e.g., effect of age, weight, menstrual status) are considered by the investigator, the neuropsychopharmacologic challenge can be a valuable strategy with which to investigate functions of the brain neurotransmitter system in humans with behavioral and neuropsychiatric disorders and to explore possible clinical correlation in terms of familial factors, clinical outcome, and treatment response.

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