Serotonin and Stress

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Forty-five years after its discovery, brain serotonin (5-HT) is still the subject of intense research aimed at understanding its role in stress adaptation. At the presynaptic level, numerous stressors increase nerve firing and extracellular 5-HT at the level of serotonergic cell bodies or nerve terminals. Different studies have reported stressor- and region-specific changes in extracellular 5-HT, a view challenged by electrophysiological and neurochemical evidence for a nonspecific response of serotonergic neurones to stressors when activity/arousal is taken into account. In addition, early studies indicate that stress-induced elevation in 5-HT synthesis, a key counter-regulatory process allowing serotonergic homeostasis, is mediated by specific neuroendocrine mechanisms. In addition to the multiplicity of postsynaptic 5-HT receptors and their specific regulation by corticoids, specificity to stressors is also underscored when considering one receptor type such as the $5\text{-}HT_{1A}$ receptor. Stress studies should consider the past experience and the genetic status of the individual as key modulators of the serotonergic responses to stress.   [Neuropsychopharmacology 21:28S–32S, 1999]   © 1999 American College of Neuropsychopharmacology. Published by Elsevier Science Inc.

Since the discovery of serotonin (5-hydroxytryptamine, 5-HT) in the mammalian brain (Twarog and Page 1953), the effects of stress upon serotonergic systems have been the focus of permanent research (Anisman and Zacharko 1982; Chaouloff 1993; Rueter et al. 1997). An overview of such research provides a reliable illustration of the progress made in that field. Early studies from the 1960s focused on the effects of stressors on brain 5-HT levels and synthesis. In the 1970s, drugs rendering possible the estimation of 5-HT turnover allowed a more detailed analysis of time- and region-dependent changes in serotonergic systems of stressed animals; in addition, reports regarding the influence of stressors upon the supply of tryptophan to the brain were first published during that period. In the 1980s, the recognition of the first subtypes of 5-HT receptors led to studies aimed at measuring the impact of acute and repeated stressors on these targets; in parallel, key studies appeared that were devoted to (1) the roles of stress hormones (e.g., corticoids) in the control of central serotonergic activity, and (2) the effects of stressors on 5-HT nerve firing. Currently, numerous studies explore the effects of stressors on extracellular 5-HT levels (as assessed by the microdialysis technique) and 5-HT receptor-coupled second messengers.

An overview of 30 years of research on stress and 5-HT indeed favors the hypothesis that numerous components of central serotonergic systems are sensitive to stressors. This result would thus fit, at first glance, with therapeutic data in anxious and/or depressed patients, data suggesting that 5-HT is an important component of the central network that provides adaptation to stress. However, among the questions that remain regarding the role of 5-HT in this network, one is of particular importance: is 5-HT a fine modulator or an on-or-off switch? If the former proposal is correct, one would expect, among other criteria, that 5-HT responds specifically to certain stressors, both in terms of dura-

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tion and location, but not (or differently) to others. With this question in mind, this article tries to delineate the effects of stress on key components of central serotonergic systems.

5-HT INNERVATION AND STRESS

When exposed to a stressor, the organism responds by two means, respectively behavioral and neuroendocrinological in nature, with the latter response aimed at providing metabolic and cardiovascular adjustments (through the sympathetic nervous system and the corticotropic axis) that allow an appropriate behavioral response to the stimulus. Indeed, there is extensive evidence for the presence of 5-HT nerve terminals and/or 5-HT receptors in key stress-related neuroendocrine (e.g., hippocampus, hypothalamus, brainstem, medulla, etc.) and behavioral (e.g., amygdala, striatum, hippocampus, cortex, periaqueductal grey, etc.) regions (Chaouloff 1993; Graeff 1993). Moreover, serotonergic cell bodies in the raphe nuclei receive afferents from a vast array of regions (Jacobs and Azmitia 1992), including those mentioned above, thus allowing 5-HT nerves to receive permanent information during stress. That immediate early gene expression, such as that of c-fos, is detected in these nuclei following different types of stressors (e.g., forced swim or restraint, Cullinan et al. 1995) illustrates the latter statement.

5-HT NERVE CELL FIRING AND STRESS

Electrophysiological studies in cat rostral raphe nuclei have indicated that the activity of dorsal raphe nucleus (DRN) serotonergic neurons is affected by a variety of metabolic, psychological, or physical stressors. However, in line with early findings indicating that the firing rate of these neurons vary positively with activity/arousal, it was reported that none of the stressors affect specifically serotonergic DRN neurons when activity/arousal was taken into consideration (Jacobs and Fornal 1991; Jacobs and Azmitia 1992). Thus, data related to serotonergic DRN neurons point to a stimulatory, albeit nonspecific, effect of stressors on forebrain/midbrain 5-HT nerve cell firing.

When placed in the framework of adaptation, these results lead to one key question: would such a nonspecificity apply to repeated or chronic stressors? Moreover, in keeping with experimental evidence for emotionality not being one unique behavioral entity but the sum of multiple independent behavioral dimensions (Ramos and Mormède 1998), the hypothesis that activity/arousal does have a stressor-dependent behavioral significance would then confer, if true, a specificity to the 5-HT nerve firing response to the stressor. Lastly, that

the aforementioned experiments were conducted in cats raises the possibility of different results in other species.

EXTRACELLULAR 5-HT AND STRESS

In a recent review of the effects of different physical, metabolic, psychological, or immunological stressors on extracellular 5-HT levels (Rueter et al. 1997), one was able to observe that extracellular 5-HT rose in most regions (1) whatever the nature of the stressor applied, and (2) without any dichotomy between nerve terminals derived from the DRN on the one hand, and the median raphe nucleus (MRN) on the other hand. In keeping with the electrophysiological data mentioned above, it was proposed that such a generalized increase in extracellular 5-HT levels was primarily determined by the level of activity/arousal displayed during stress (Rueter et al. 1997). Actually, the reports of positive correlations between extracellular 5-HT levels and activity/arousal support the aforementioned hypothesis (Rueter et al. 1997). However, recent studies have shown that injection of either lipopolysaccharide (an immunological stressor) or corticotropin-releasing factor (a key transmitter during stressful events) elicits changes in extracellular 5-HT levels and locomotor activity that do not correlate among each other (Linthorst et al. 1995; Price et al. 1998).

That almost all stressors have an overall stimulatory effect on extracellular 5-HT levels would argue against a specific effect of stress on presynaptic 5-HT tone (whether activity/arousal is taken into account or not). However, this view has been recently challenged by some studies claiming stressor- and site-dependent changes in extracellular 5-HT levels (Adell et al. 1997; Kirby et al. 1995, 1997), including within raphe nuclei (Adell et al. 1997). Moreover, site-dependent changes in extracellular 5-HT responses to a given stressor may also be measured by altering the context (including stressor controllability, Amat et al. 1998) within which the stressor is applied (Wilkinson et al. 1996).

To add to the aforementioned uncertainties as to the specificity vs. nonspecificity of the presynaptic serotonergic response to stress, the following issues should be taken into account. First, any direct comparison between all these studies is rendered impossible due to key experimental differences that include the addition (or not) of a 5-HT reuptake inhibitor to allow an estimation of extracellular 5-HT levels, the time period (light vs. dark) during which the experiment was run, the recovery period allowed between implantation of the cannula and stress exposure, the rat strain, prior housing (single vs. collective) before housing the rat alone in the experimental bowl, etc. Second, the microdialysis technique is not fine enough to distinguish between cell

clusters. This limit is of importance when considering nuclei such as the DRN which is made of different subdivisions, each one receiving specific afferent inputs (Peyron et al. 1998). Practical limits also concern the analytical procedure (liquid chromatography and electrochemical detection) that allows the estimation of 5-HT levels: thus, these are based on 20-minute samples, thereby impeding any fine time-dependent observation of early events, if any. Third, any discussion regarding the relationships between nerve-firing activity and extracellular 5-HT may well be superfluous given that none of the studies examined the mechanisms underlying stress-elicited increases in extracellular 5-HT. Thus, besides the fact that neuronal, as well as glial reuptake of 5-HT may be altered during stress, the potential influence of presynaptic heteroreceptors cannot be dismissed. Moreover, it could be that specificity to a given stressor in a given nucleus is provided by local mechanisms (as illustrated for adrenocorticotropic hormone secretion, Romero and Sapolsky 1996), such as cotransmitters, local peptides (e.g., 5-HT-moduline, Massot et al. 1996), or carrier-mediated release. The high ratio of the number of serotonergic varicosities over the number of serotonergic nerve fibers (Jacobs and Azmitia 1992) would speak in favor of such a local specificity. Lastly, it should be remembered that the measurement of extracellular 5-HT does not distinguish between intrasynaptic and extrasynaptic (paracrine) transmission (as measured by fast-scan cyclic voltammetry, Bunin and Wightman 1998), the ratio of the first over the second being possibly altered in a stress- and region-specific manner.

5-HT SYNTHESIS/METABOLISM AND STRESS

Numerous stressors increase 5-HT synthesis/turnover (Chaouloff 1993), a result which could, at first glance, reinforce the belief that the response of serotonergic systems to stressors is nonspecific. Although none of the studies examined how each stressor affected activity/arousal, it is noteworthy that different stressors have been shown to promote stressor- and region-dependent changes (Lee et al. 1987; Petty et al. 1997). Moreover, there is some specificity regarding the mechanisms (i.e., increased tryptophan availability, tryptophan hydroxylase hyperactivity) through which stress increases 5-HT synthesis/turnover (Chaouloff 1993). For example, exposure to a metabolic stressor (insulin injection in fasted rats), a physical stressor (acute running), or a psychological stressor (immobilization) leads to pronounced increases in brain tryptophan availability by means of stressor-specific mechanisms (Chaouloff 1993). Regarding tryptophan hydroxylase, there is evidence for a key regulatory, albeit permissive, role of corticoids (Chaouloff 1993). Thus, sound stress increases tryptophan hydroxylase activity through glucocorticoid receptors, an effect prevented by adrenalectomy (Singh et al. 1990). On the other hand, adrenalectomy does not affect footshock-induced rises in 5-HT synthesis/metabolism (Dunn 1988), thereby suggesting again some specificity with regard to the nature of the stimulus. Because stress-induced increases in extracellular 5-HT precede largely those in 5-HT synthesis, it is likely that the latter increases only serve to counterbalance neuronal depletion of 5-HT due to release (Anisman and Zacharko 1982; Chaouloff 1993). If true, such a hypothesis indicates that homeostasis within serotonergic systems, as provided by counter-regulatory stress hormones, is a stressor-specific event.

5-HT$_{1A}$ RECEPTORS AND STRESS

It is likely that the specificity of the serotonergic responses to stress is provided by the multiplicity of pre- and postsynaptic 5-HT receptor types. Actually, some receptors, but not others, display sensitivity to stressors (Chaouloff 1993), possibly through the actions of corticosteroids (Chaouloff 1995). There is also some evidence for one receptor type being specifically affected by stressors. In keeping with the key role of 5-HT$_{1A}$ autoreceptors on nerve firing activity and/or release of 5-HT, some studies have analyzed these receptors in stressed animals. Actually, the sensitivity of DRN 5-HT$_{1A}$ autoreceptors (but not their numbers) was decreased by a stress of long, but not short, duration (novel environment, Laaris et al. 1997). This result is in keeping with the large delay observed between application of corticosterone and the resulting changes in 5-HT$_{1A}$ autoreceptor sensitivity (Laaris et al. 1995). This suggests that stress-induced decreases in 5-HT$_{1A}$ autoreceptor sensitivity appear later, possibly to allow effective synthesis of the amine without any feedback interference.

Acute stress either increases or decreases hippocampal and/or cortical 5-HT$_{1A}$ receptor binding in a stressor- and subregion-dependent manner (Raghupathi and McGonigle 1997), providing support for specificity. This result is noteworthy given the belief that stressors need to be administered repeatedly to observe a (glucocorticoid-dependent) decrease in 5-HT$_{1A}$ receptor binding, especially in the hippocampus (Flügge 1995, but see Mendelson and McEwen 1991). Regarding specificity on chronic stress, 5-HT$_{1A}$ receptor mRNA expression (and binding) changes have been shown recently to depend on the severity and/or the predictability of the stressor (Lopez et al. 1998; McKittrick et al. 1995). Future studies on recently discovered 5-HT receptors (e.g., 5-HT$_{6,7}$ receptors) including analyses of second messenger-dependent protein kinase cascades (Duman et al. 1997) could provide further biochemical evidence for specific reactivity to stressors.

DETERMINANTS OF 5-HT RESPONSES TO STRESS

In a review of the neurobiological correlates of suicidal behavior within a stress-diathesis model, noradrenergic systems were viewed as key targets of stress whereas serotonergic systems would be linked, as a biochemical trait, to risk factors and vulnerability (Mann 1998). Among these risk factors, the genetic profile of the individual and his early experiences are key components that promote suicide in some depressed persons but not in others (Mann 1998). Initial studies on the effects of stressors upon serotonergic systems stem from the hypothesis that stress is an etiological factor in mood disorders, especially depression. Our belief is that this hypothesis needs to integrate to a larger extent the aforementioned risk factors. The following examples provide an illustration of how the serotonergic responses to stressors may depend on past experience and genetics, thereby providing additional elements in the debate regarding the specificity of 5-HT reactivity to stress. First, in keeping with the reports that diabetic patients are prone to mood disorders, we have analyzed the effects of restraint on extracellular 5-HT levels in the hippocampus of control and diabetic rats: actually, restraint-elicited increases in extracellular 5-HT levels were not observed in diabetics, despite rises in corticosterone levels and tissue 5-HIAA (Thorre et al. 1997). Second, an early environmental change such as isolation rearing has been shown to promote a rise in extracellular 5-HT in the nucleus accumbens in response to inescapable footshocks or context conditioning (Fulford and Marsden 1998). Lastly, in a genetic study comparing the psychoneuroendocrine effects of repeated social defeat in anxious (Lewis) and non-anxious (SHR) rats, it was observed that beside identical changes in some serotonergic targets (e.g., hippocampal 5-HT_{1A} receptors), cortical 5-HT_{2A} receptors increased in Lewis rats only, a change associated with a strain-selective anxiogenic effect of stress (Berton et al. 1998). These examples underscore the relevance of animal models differing in their past experience and/or genetic profiles to study the influence of risk factors on the reactivity of central serotonergic systems to stress.

CONCLUSIONS

This rapid survey has tried to summarize some experimental findings which, taken together, strengthen the hypothesis that central serotonergic transmission is affected during stress. Although there are anatomical, electrophysiological, and biochemical findings in support of that contention, there is still a debate as to the role of 5-HT during stress. Thus, some arguments favor the hypothesis that 5-HT acts as a fine modulator during stress whereas others support the view that 5-HT is grossly affected by stressors, possibly in relation to the activity/arousal level of the individual. Actually, two main limits are to be encompassed before any clear-cut answer may be provided. The first is technological, as illustrated by the limits of the microdialysis technique, which has helped to get important insights in the relationships between stress and extracellular 5-HT, but whose sensitivity is insufficient to answer some crucial questions related to rapid changes in discrete areas of the brain. New technologies such as fast-scan cyclic voltammetry should be helpful in stress studies. The second limit relates to the animal models used, which should first use appropriate controls to detect the impact of activity/arousal in the effects of stress on 5-HT, but also take into account both the past experience and the genetic status of the individual.

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