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CELLULAR MECHANISMS UNDERLYING THE DEVELOPMENT AND EXPRESSION OF INDIVIDUAL DIFFERENCES IN THE HYPOTHALAMIC–PITUITARY–ADRENAL STRESS RESPONSE

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Summary—Several years ago Levine, Denenberg, Ader, and others described the effects of postnatal "handling" on the development of behavioral and endocrine responses to stress. As adults, handled rats exhibited attenuated fearfulness in novel environments and a less pronounced increase in the secretion of the adrenal glucocorticoids in response to a variety of stressors. These findings clearly demonstrated that the development of rudimentary, adaptive responses to stress could be modified by environmental events. We have followed these earlier studies, convinced that this paradigm provides a marvellous opportunity to examine how subtle variations in the early environment alter the development of specific neurochemical systems, leading to stable individual differences in biological responses to stimuli that threaten homeostasis. In this work we have shown how early handling influences the development of certain brain regions that regulate glucocorticoid negative-feedback inhibition over hypothalamic–pituitary–adrenal (HPA) activity. Specifically, handling increases glucocorticoid (type II corticosteroid) receptor density in the hippocampus and frontal cortex, enhancing the sensitivity of these structures to the negative-feedback effects of elevated circulating glucocorticoids, and increasing the efficacy of neural inhibition over ACTH secretion. These effects are reflected in the differential secretory pattern of ACTH and corticosterone in handled and nonhandled animals under conditions of stress.

In more recent years, using a hippocampal cell culture system, we have provided evidence for the importance of serotonin-induced changes in cAMP levels in mediating the effect of postnatal handling on hippocampal glucocorticoid receptor density. The results of these studies are consistent with the idea that environmental events in early life can permanently alter glucocorticoid receptor gene expression in the hippocampus, providing evidence for a neural mechanism for the development of individual differences in HPA function.

THE ADRENOCORTICAL RESPONSE TO STRESS

The hypothalamic–pituitary–adrenal (HPA) axis, as described by Selye [1], is highly responsive to stress, and the release of the adrenal glucocorticoids under conditions which threaten homeostasis represents one of the central adaptive mechanisms among mammals. The secretion of corticotropin-releasing hormone (CRH) and co-secretagogues such as vasopressin (AVP) and oxytocin into the portal system of the anterior pituitary during stress causes an increase in the release of adrenocorticotropin (ACTH) into circulation [e.g. 2–9]. The elevated ACTH levels stimulate an increase in the release of adrenal glucocorticoids. The highly catabolic glucocorticoids produce lypolysis, increasing the level of free fatty acids, glycogenolysis, increasing blood glucose levels, and protein catabolism, which increases amino acid availability as substrates for gluconeogenesis, further increasing blood glucose levels [10, 11]. Together, these actions assist the organism under stressful conditions, in part at least, by increasing the availability of energy substrates. The glucocorticoids also suppress immunological responses [11], protecting against the occurrence of inflammation at a time when mobility may be important to the animal.

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However, continued exposure to elevated glucocorticoid levels can present a serious risk for the organism. In addition to a general suppression of anabolic processes, prolonged glucocorticoid exposure can lead to muscle atrophy, decreased sensitivity to insulin and a risk of steroid-induced diabetes, hypertension, hyperlipidemia, hypercholesterolemia, arterial disease, amenorrhea, and impotency, and the impairment of growth and tissue repair, as well as immunosuppression [10, 11]. Thus, once the stressor is terminated, it is very clearly in the animal's best interest to "turn off" the HPA stress response and the efficacy of this process is determined by the ability of the glucocorticoids to inhibit subsequent ACTH release (i.e. glucocorticoid negative-feedback).

Circulating glucocorticoids feedback onto the pituitary and specific brain regions to inhibit the ACTH from the anterior pituitary [12–17]. In addition to the more obvious target sites, such as the pituitary and the medial–basal hypothalamus, there is now considerable evidence for the importance of extrahypothalamic regions in the inhibition of HPA activity [12]. Most notable among these regions is the hippocampus [18]. Hippocampal lesions or ablations are associated with elevated corticosterone levels under both basal, stress, and post-stress conditions [e.g. 19–23]. Moreover, hippocampectomized animals show reduced suppression of ACTH following exogenous glucocorticoid administration [19] and increased CRH and AVP mRNA levels in the paraventricular n. of the hypothalamus [24]. These findings, together with the fact that the hippocampus is rich in corticosteroid receptors [25], suggest that this structure is involved in the inhibitory influence of glucocorticoids over adrenocortical activity.

Evidence from a number of models suggests that a decrease in hippocampal corticosteroid receptor density is associated with a hypersecretion of corticosterone both under basal conditions and following the termination of stress (i.e. less effective negative-feedback). There are decreased levels of hippocampal corticosteroid receptors binding in the aged rat [26–32], lactating rats [33], and immature rats [34–38] and these animals also hypersecrete corticosterone [39–41]. Perhaps the most impressive evidence comes from studies with the AVP-deficient, Brattleboro rat [22]. These animals show a deficit in corticosteroid receptors in the hippocampus and pituitary and hypersecrete corticosterone following stress. The hippocampal receptor deficit is reversed with AVP treatment and, so long as the treatment is continued, receptor levels remain elevated and the animals exhibit normal corticosterone secretion following stress [22].

The uptake of corticosterone in rat brain is associated with at least two distinct types of corticosteroid receptors [e.g. 42–52]. The mineralocorticoid (or type I) receptor binds *in vitro* to both corticosterone and the mineralocorticoids, aldosterone and RU 26752, with high affinity, and binds the synthetic glucocorticoid, RU 28362, with very low affinity. The glucocorticoid (or type II) receptor is far more diffusely distributed throughout the brain, and binds corticosterone, dexamethasone, and RU 28362 with high affinity, and RU 26752 and aldosterone with lower affinity. Although both receptors bind corticosterone with high affinity, the K_d of the mineralocorticoid receptor for corticosterone ($\sim$0.5 nM) is lower than that of the glucocorticoid receptor ($\sim$2.0–5.0 nM; e.g. [46]).

A physiological consequence of this difference in affinity for corticosterone is the fact that these receptors then show different rates of occupancy under basal corticosterone levels. About 80–90% of the mineralocorticoid sites are occupied under basal corticosterone levels [46, 48, 49], rendering the hippocampal mineralocorticoid receptor relatively insensitive to dynamic variations in corticosterone levels. In contrast, the glucocorticoid receptor is highly responsive to dynamic changes in corticosterone titers, such as those occurring during stress [46, 48, 49, 54]. Under conditions of basal circulating corticosterone only about 10–15% of the glucocorticoid receptors are occupied. Stress results in a dramatic increase in the hormone-receptor signal, such that immediately following a 20 min period of immobilization about 75% of glucocorticoid receptors are occupied. Corticosterone injections (15 mg/kg) that mimic the steroid levels seen during stress also result in about 75% occupancy of glucocorticoid receptors. These findings, together with the known negative-feedback efficacy of the synthetic corticoids such as dexamethasone, once thought to selectively bind to the glucocorticoid receptor, suggested that it was this site and not the mineralocorticoid-like receptor, that underlies the negative feedback actions of glucocorticoids under post-stress conditions.

However, Dallman and her colleagues have recently provided evidence for the involvement

of both mineralocorticoid and glucocorticoid receptors in the regulation of ACTH levels in rats. In these studies hippocampal implants of both the glucocorticoid receptor antagonist, RU 38486, and the mineralocorticoid receptor antagonist, RU 26752, resulted in elevated levels of plasma ACTH [55]. Moreover, Ratka *et al.* [56] have found that systemic injections of either antagonist resulted in elevated post-stress corticosterone levels in intact rats. These findings suggest that glucocorticoid negative-feedback may involve both mineralocorticoid and glucocorticoid receptor sites.

THE EFFECT OF HANDLING ON THE ADRENOCORTICAL RESPONSE TO STRESS

The handling procedure usually involved removing rat pups from their cage, placing the animals together in small containers, and 15–20 min later, returning the animals to their cage and their mothers. The manipulation was performed daily for the first 21 days of life. In response to a wide variety of stressors handled (H) rats secrete less corticosterone and show a faster return to basal corticosterone levels following the termination of stress than do nonhandled (NH) animals [26, 57–63]. These differences are apparent as late as 24–26 months of age [26, 28], indicating that the handling effect persists over the entire life of the animal. The differences in HPA function are not due to changes in adrenal sensitivity to ACTH [64] or in pituitary sensitivity to CRH [63]. Moreover, there are no differences between H and NH animals in the metabolic clearance rate for corticosterone [63, 64]. Rather, the difference lies in the fact that the NH animals show increased secretion of corticosterone both during and following stress.

Young adult H and NH animals do not differ in levels of corticosteroid-binding globulin (CBG), the principle plasma binder for corticosterone [27, 62] or in free corticosterone levels [27]. This finding is of considerable importance since brain uptake of corticosterone appears to approximate the nonCBG bound (free + albumin-bound) portion of the steroid [65]. Thus, differences in total corticosterone are likely predictive of differences in brain uptake of the steroid. One further point of importance concerns the specificity of this difference. Young adult H and NH animals do not differ in basal corticosterone levels at any time point over the diurnal cycle [27, 63]. Thus, the difference between the two groups of animals is specific to conditions of stress and is not seen under normal, resting conditions. This finding also indicates that differences in HPA activity observed during stress cannot be accounted for by differences in pre-stress, basal glucocorticoid levels.

H and NH animals also differ in plasma ACTH and ACTH secretagogue responses to stress. Levels of CRF-like bioactivity [66] and plasma ACTH [63] are higher both during and following stress in NH animals. These findings suggest that the mechanism(s) for differences between H and NH animals is located above the level of the pituitary and that the post-stress difference, at least, may be related to differential sensitivity of CNS negative-feedback processes.

THE EFFECT OF HANDLING ON HPA NEGATIVE-FEEDBACK PROCESSES

On the basis of the relative hypersecretion of ACTH and corticosterone by NH animals, we wondered whether H and NH animals might differ in negative-feedback sensitivity to circulating glucocorticoids. We [63] tested this idea using a classical negative-feedback paradigm based on the finding that high levels of circulating glucocorticoids feedback onto the brain and/or pituitary to inhibit subsequent HPA activity [12–14, 17]. Such delayed, negative-feedback persists for hours following exposure to elevated glucocorticoid levels [14]. In these experiments, H and NH animals were injected with one of five doses of either corticosterone or dexamethasone 3 h prior to a 20-min immobilization stress. Both glucocorticoids were more effective in suppressing stress-induced HPA responses in the H animals (i.e. the ID_{50} for both corticosterone and dexamethasone was 5–10 times lower in the H animals). These data suggest that indeed the H animals are more sensitive to the negative-feedback effects of circulating glucocorticoids on HPA activity.

Since this delayed form of negative-feedback is likely mediated by the binding of corticosterone to soluble intracellular receptors, we have measured both mineralocorticoid and glucocorticoid receptor sites in selected brain regions and pituitary of young adult H and NH animals [26–28, 38, 63, 67–69]. The results of these studies have demonstrated that there are significant and regionally-specific differences in glucocorticoid receptor binding capacity as a function of handling. H animals show increased

glucocorticoid receptor binding capacity in the hippocampus, but not in the septum, amygdala, hypothalamus, or pituitary. The difference in the receptor binding data is clearly related to the number of receptor sites, and not to the affinity of the receptor for [^{3}H]radioligand, RU 28362. Moreover, the difference occurs in glucocorticoid receptors, but not the mineralocorticoid receptor sites (measured using either radiolabeled aldosterone or corticosterone + 50-fold excess of cold RU 28362).

In a recent experiment we [63] have demonstrated that this difference in hippocampal glucocorticoid receptor density is related to the more efficient suppression of post-stress HPA activity in the H animals. Chronic administration of corticosterone results in a 30–45% down-regulation of hippocampal glucocorticoid receptor binding sites [36, 70, 71]. The effect is highly specific to the hippocampus, such that receptor binding capacity in the hypothalamus and pituitary are unaffected. In this experiment we treated H animals for 5 days with corticosterone, and allowed two days for steroid clearance. Hippocampal glucocorticoid receptor density was down-regulated in the H + corticosterone animals to levels that were indistinguishable from those of NH animals, and significantly less than that of the H + vehicle animals. There were no differences in glucocorticoid receptor density in the hypothalamus or pituitary. When the animals in these groups were exposed to a 20-min immobilization stress, we found that the H + corticosterone animals, like the NH animals, hypersecreted corticosterone 60 and 120 min post-stress in comparison to the H + vehicle, control animals. These data suggest that the difference in negative-feedback efficiency between H and NH is related to the differences in hippocampal glucocorticoid receptor density. Thus, the chronic corticosterone treatment reversed both the handling-induced increase in hippocampal glucocorticoid receptor binding capacity and the difference in post-stress HPA activity. It appears, then, that the increase in glucocorticoid receptor sites in the hippocampus is a critical feature for the handling effect on HPA function. The increase in receptor density appears to increase the sensitivity of the hippocampus to circulating glucocorticoids, enhancing the efficacy of negative-feedback inhibition over HPA activity, and serving to reduce post-stress secretion of ACTH and corticosterone in H animals.

The effect of post-natal handling on HPA negative-feedback likely involves glucocorticoid receptor differences in at least one other region. Handling also increases glucocorticoid receptor density in the frontal cortex [68]. We have recently provided evidence for the role of the frontal cortex in the regulation of stress-induced HPA activity [72]. Ablations of the medial prefrontal cortex produce increased levels of both ACTH and corticosterone both during and following the termination of stress. Corticosterone implants directly into this region produce a 40–50% decrease in stress-induced ACTH and corticosterone levels. Interestingly, these effects are apparent only with more moderate (neurogenic?) stressors, in this case restraint. Neither ablations of the medial prefrontal cortex nor corticosterone implants into this region had any effect on ACTH or corticosterone levels observed using ether stress, a more severe stressor associated with 2–3 times higher levels of ACTH. Moreover, these effects were observed only during or following stress; neither treatment altered basal ACTH or corticosterone levels at any point over the diurnal cycle. These findings suggest that the handling effect on HPA function might well involve altered glucocorticoid receptor density in the frontal cortex.

THE EFFECT OF HANDLING ON HPA ACTIVITY DURING STRESS

We have focused largely on the mechanisms underlying the differences in post-stress levels of plasma ACTH and corticosterone. However, the majority of the earlier studies on handling were directed at the differences in corticosterone levels between H and NH rats achieved during stress. In general, H animals secreted lower levels of corticosterone during stress. However, this effect was often dependent upon the gender of the animals [1, 75]. Likewise, we have consistently found that H and NH female rats do not differ in corticosterone levels during stress [28, 62]. Females, like males, differ in post-stress corticosterone levels and in hippocampal glucocorticoid receptor binding [28, 62, 68]. These findings suggest that differences in hippocampal corticosteroid receptor density are probably not related to differences in HPA activity during stress.

The results of an earlier study have implicated an entirely different receptor system in the effect of handling on HPA activity during stress. Handling results in decreased levels of pituitary

transcortin receptor levels in male, but not female rats. The transcortin receptor is an intracellular (or at least a soluble receptor) that is physico–chemically identical to plasma CBG. Like CBG, transcortin binds with high affinity to corticosterone and appears to act as a buffer, reducing the binding of corticosterone to classical corticosteroid receptors and thus the nuclear hormone-receptor signal. Sakly and Koch [74, 75] have found that transcortin levels in neonatal rats are very low and are associated with increased nuclear uptake of corticosterone. This increased corticoid signal appears to be associated with enhanced glucocorticoid negative-feedback over pituitary ACTH release [40, 76] and may, in part, account for the apparent reduced ability of the neonate to secrete ACTH in response to stress (the so-called stress hyporesponsive period). As the animal matures, transcortin levels rise, reducing nuclear uptake of corticosterone and dampening the negative-feedback effect on ACTH. Thus, with age the HPA response emerges and its appearance is correlated with the changes in pituitary transcortin receptor binding (as well as other features of the system [40, 77]).

The reduced pituitary transcortin levels in the H male rats might allow for an increase in the nuclear uptake of corticosterone in the pituitary. Such an effect would be expected to reduce ACTH secretion during stress compared to NH rats, and this is indeed the common finding. It is, of course, interesting that H and NH female rats differ in neither HPA activity during stress nor in pituitary transcortin levels. We are currently examining the hypothesis that differences in stress-induced levels of ACTH release might be related to the effect of handling on pituitary transcortin levels. Regardless of the merit of this hypothesis, these findings remind us that the individual variation in HPA function is likely to involve a number of mechanisms situated at various levels of the axis [78, 79].

THE MECHANISM OF ACTION OF HANDLING ON GLUCOCORTICOID RECEPTOR DEVELOPMENT

In our initial studies, animals were handled for the first 21 days of life. Based on the earlier work by Levine, we wondered whether some portion of this 3-week period might represent a critical period for the handling effect on glucocorticoid receptors. In one study [67], animals were handled daily and sacrificed on either post-natal day 3, 7, 14, or 21 of life. We found that, in comparison to same-aged NH animals, H animals exhibited significantly increased hippocampal glucocorticoid receptor density as early as day 7 of life and that the magnitude of the effect did not increase thereafter. We also found that handling between days 1 and 7 of life was as effective in increasing hippocampal glucocorticoid receptor density as handling over the entire first 3 weeks. Handling over the second week of life (i.e. between days 8 and 14) was somewhat less effective, whereas animals handled between days 15 and 21 did not differ from NH animals in glucocorticoid receptor binding. Thus, glucocorticoid receptor binding capacity appears to be especially sensitive to environmental regulation during the first week of life.

This temporal pattern corresponds to the normal developmental changes in glucocorticoid receptor density occurring over the early post-natal life [34–38]. Glucocorticoid receptor density is low on post-natal day 3 ($\sim$30% of adult values) and increases steadily towards adult values which are achieved by about the third week of life. It is during this period of ontogenic variation that environmental events can influence the development of the receptor population. In contrast to the glucocorticoid receptor, mineralocorticoid receptor density does not vary significantly with age. Hippocampal mineralocorticoid receptor density in early post-natal life is indistinguishable from that of adult rats [37, 38] and, as mentioned above, handling has no effect on hippocampal mineralocorticoid receptor binding [38]. Thus, it is tempting to consider the relationship between the developmental pattern in hippocampal glucocorticoid receptor density and (1) the sensitivity of this receptor system to environmental stimuli, and (2) the timing of the critical period for these stimuli on glucocorticoid receptor development. However, the developmental pattern for glucocorticoid receptor binding in regions not affected by handling, such as the hypothalamus, amygdala, and septum [34–36], is identical to that of the hippocampus and the frontal cortex [80]. Thus, it is unlikely that the handling effect on glucocorticoid receptor density in the hippocampus and the frontal cortex can be explained simply by the immature status of the glucocorticoid receptor system during the first weeks of life.

Handling during the first week of life can involve a mild and transient drop in the pup's body temperature [81]; although this feature of

the manipulation is not critical for the effect on HPA activation during stress [77]. This is likely associated with the relatively immature thermoregulatory abilities of young rats over the first 2 weeks of life. Such changes in body temperature and/or some other sensory component of the handling manipulation activate the hypothalamic–pituitary–thyroid axis, leading to increased levels of circulating thyroxine (T_4) and increased intracellular levels of the biologically more potent T_4 metabolite, triiodothyronine (T_3). Handling results in a modest, but reliable ($\sim$30%) increase in plasma thyroid hormones (Meaney and Aitken, unpublished). Thus, we [69] examined whether the effects of handling might be mediated by increased exposure to these thyroid hormones. Neonatal rat pups were exposed to elevated levels of either T_4, T_3, or the vehicle over the first week of life. Both T_4 and T_3 treatment resulted in significantly increased glucocorticoid receptor binding capacity in the hippocampus in animals examined as adults. Like the handling manipulation, neither T_4 nor T_3 treatment affected hypothalamic or pituitary glucocorticoid receptor density. Moreover, administration of the thyroid hormone synthesis inhibitor, propylthrouracil (PTU), to H pups for the first 2 weeks of life completely blocked the effects of handling on hippocampal glucocorticoid receptor binding capacity. These data suggest that indeed the thyroid hormones might mediate, in part at least, the effects of neonatal handling on the development of the hippocampal glucocorticoid receptor system.

Systemic injections of neonatal rat pups represent a fairly crude manipulation and while these data might implicate the thyroid hormones, there is no indication that the hippocampus is the actual critical site of action for thyroid hormone effect. In order to examine whether thyroid hormones might act directly on hippocampal cells we have turned to an *in vitro* system, using primary cultures of dissociated hippocampal cells [82]. The hippocampal cells are taken from embryonic rat pups (E20) and beginning on the fifth day after plating the cultures were exposed to 0, 1, 10, or 100 nM T_3. The cells were cultured in 10% fetal calf serum, stripped of thyroid hormones. Thus far, the results of several experiments have failed to detect any effect of thyroid hormones on glucocorticoid receptor density in cultured hippocampal cells (Meaney, unpublished). These *in vitro* data suggest that (a) the effects of the thyroid hormones on the glucocorticoid receptor binding capacity occurs at some site distal to the hippocampus cells or (b) thyroid hormones interact at the level of the hippocampus with some other hormonal signal that is obligatory for the expression of the thyroid hormone effect.

Thyroid hormones have pervasive effects throughout the developing CNS and one such effect involves the regulation of central serotonergic neurons [e.g. 83]. Thyroid hormones increase serotonin (5-HT) turnover in the hippocampus of the neonatal rat [84]. Handling also increases hippocampal 5-HT turnover, and thus both manipulations increase serotonergic stimulation of hippocampal neurons. There is also direct evidence for an effect of 5-HT on glucocorticoid receptor density in the neonatal rat.

5,7-Dihydroxytryptamine (5,7-DHT) lesions of the raphe 5-HT neurons dramatically reduce the ascending serotonergic input into the hippocampus. Rat pups administered 5,7-DHT on day 2 of life showed reduced hippocampal glucocorticoid receptor density as adults [84]. Similar lesions of ascending catecholaminergic systems, using 6-hydroxydopamine, had no effect on the development of glucocorticoid receptor density in the hippocampus (Meaney, unpublished). Interestingly, this effect may also be specific to the neonatal period, since similar chemical lesions of the ascending 5-HT neurons in adult animals do not appear to affect hippocampal glucocorticoid receptor binding capacity (M. Lowry, personal communication). Finally, we found that effects of post-natal handling on glucocorticoid receptor binding are blocked by concurrent administration of the 5-HT_2 receptor antagonist, ketanserin [84].

Recent studies [85] of 5-HT turnover have provided some insight into why the hippocampus and frontal cortex are selectively affected by handling. We found that when rat pups were handled for the first seven days of life, and sacrificed immediately following handling on day 7, that 5-HT turnover was significantly increased in the hippocampus and frontal cortex, but not in the hypothalamus or amygdala (regions where handling has no effect on glucocorticoid receptor density). These data suggest that handling selectively activates certain ascending 5-HT pathways and that it is this feature of the handling effect, together with the existence of an immature glucocorticoid receptor system, that underlies the sensitivity of this receptor system in specific brain regions to

regulation by environmental events during the first weeks of life.

The next question then, is whether 5-HT might be mediating the effects of handling at the level of the hippocampal cells. We have recently found that 5-HT has a profound effect on glucocorticoid receptor density in cultured hippocampal cells [86]. In hippocampal cells cultured in the presence of 10 nM 5-HT there was a two-fold increase in glucocorticoid receptor binding. The effect of 5-HT was dose-related, with an ED_{50} of 4.3 nM, and required about 4 days for the maximal effect to occur. Shorter periods of exposure are virtually without effect, suggesting that the effect of 5-HT may involve the increased synthesis of receptors. Moreover, the effect of 5-HT on glucocorticoid receptor binding appears to occur uniquely in the neuronal cell population. We found that there was no effect of 5-HT on glucocorticoid receptor binding in hippocampal glial-enriched cell cultures. This finding is not surprising, since our initial studies were performed with cultures comprised largely (~75%) of neuron-like cells [86].

There are two very intriguing features of this effect that bear directly on the question of the hippocampal cell cultures as a model for neural differentiation [87]. First, the effects of 5-HT on glucocorticoid receptor binding in hippocampal cell cultures are restricted to the first 3 weeks in culture. Thus, cultures treated with 10 nM 5-HT for 7 days at any time during the first 3 weeks in culture show a significant increase in glucocorticoid receptor density; however, the effect is lost after this point. Cultures treated with 10 nM 5-HT for 7 days during the fourth week following plating show no increase in glucocorticoid receptor binding. Second, the increase in glucocorticoid receptor binding capacity following exposure to 10 nM 5-HT persists as long as 40 days following 5-HT removal from the medium [87]. Thus, the effect of 5-HT on glucocorticoid receptor density observed in hippocampal culture cells mimics the long term effects of neonatal handling. It will be of considerable interest to understand the cellular events underlying these features of the 5-HT effect on hippocampal development.

The effects of 10 nM 5-HT on glucocorticoid receptor density in cultured hippocampal cells was completely blocked by the 5-HT_2 receptor antagonists, ketanserin and mianserin [86]. Moreover, the 5-HT_2 agonists 1-(2,5-dimethoxy-4-iodophenyl)-2-amino-propane (DOI), *m*-trifluoromethyl-phenylpiperazine (TFMPP), and quipazine were also effective in increasing glucocorticoid receptor binding in hippocampal culture. Selective agonists or antagonists of the 5-HT_{1a} or 5-HT_3 receptors had no effect on glucocorticoid receptor binding. Using [^{125}I]7-amino-8-iodo-ketanserin as the radioligand, we have confirmed the presence of high affinity 5-HT_2 binding sites in our cultured hippocampal cells. Titeler *et al.* [e.g. 88] have provided evidence that the ketanserin-labeled 5-HT_2 site may exist in two states: An agonist state (5-HT_{2H}) with a high, nanomolar affinity for 5-HT and an antagonist state (5-HT_{2L}) with a low, micromolar affinity for 5-HT. In both states the receptor shows a high affinity for antagonists, such as ketanserin. The 5-HT_{2H} site binds with high affinity to DOI, TFMPP, and quipazine, and Titeler *et al.* [88] have reported a K_d of ~5 nM for 5-HT. This K_d is a close approximation of the ED_{50} (4.3 nM) for the effect of 5-HT on glucocorticoid receptors in cultured hippocampal cells. Taken together, these findings suggest that this effect of 5-HT appears to be mediated via high-affinity, 5-HT_2 receptor.

We are currently examining the nature of the secondary messenger systems involved in this serotonergic effect on glucocorticoid receptor binding. In doing so, we [89] have found that low nanomolar concentrations of 5-HT (ED_{50} = 7.2 nM) increase cAMP levels in cultured hippocampal cells, with no effect on cGMP levels. This increase in cAMP is blocked by ketanserin and mimicked by both quipazine and DOI. Moreover, treatment with the stable cAMP analog, 8-bromo-cAMP or with 10 μM forskolin produce a significant increase in glucocorticoid receptor density in cultured hippocampal neurons. The effect of 8-bromo cAMP is concentration-related, and the maximal effect of 8-bromo-cAMP (~181%) is comparable to that for 5-HT (~195%). Interestingly, as with 5-HT, the effects of 8-bromo cAMP on glucocorticoid receptor density were not apparent until at least 4 days of treatment. The similarity in the time courses is of obvious interest, and the actual period of time involved suggests that both treatments might exert their effects through an alteration in receptor synthesis. Indeed, the effect of 10 nM 5-HT on glucocorticoid receptor density in cultured hippocampal cells is blocked by either cyclohexamide or actinomyocin D (O'Donnell and Meaney, unpublished).

Taken together, these findings suggest that changes in cAMP concentrations may mediate

the effects of 5-HT on glucocorticoid receptor synthesis in hippocampal cells. In a recent study we [89] have found that the cyclic nucleotide-dependent protein kinase inhibitor, H8 [90] completely blocked the effects of 10 nM 5-HT on glucocorticoid receptor binding in hippocampal cell cultures. In contrast, the protein kinase C inhibitor, H7, had no such effect. These data suggest that activation of protein kinase A might, at some point, be involved in the serotonergic regulation of hippocampal glucocorticoid receptor development.

CONCLUSIONS

Handling during the early post-natal period leads to increased glucocorticoid receptor binding in the hippocampus and is associated with enhanced negative-feedback control over HPA function. It is likely that this plasticity reflects a basic process, whereby the early environment is able to "fine tune" the sensitivity and efficiency of certain neuroendocrine systems that mediate the animal's response to stimuli that threaten homeostasis. Such plasticity is likely to be of considerable importance to a species like the rat, which prospers in a tremendous range of ecological niches. In this sense, it is important to note that we do not consider H or NH animals to be superior to one another. They differ as a function of the variation in early stimulation afforded the animals. What is of greatest interest to us, is the possibility that the handling manipulation provides a paradigm for the study of the molecular processes through which the development of specific neuroendocrine systems is directed by the environment. This, of course, was the source of the considerable enthusiasm for handling studies in the early periods of developmental psychobiology. With the recent advances in the molecular biology of CNS function, developmentalists are now better poised to examine in detail environment–gene interactions in the developing mammal.

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