ELSEVIER

Recent Developments in Anxiety, Stress, and Depression

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FILE, S. E. *Recent developments in anxiety, stress, and depression.* PHARMACOL BIOCHEM BEHAV 54(1) 3-12, 1996. — Recent research in the development, analysis, and pharmacology of animal tests of state anxiety is discussed, including the use of responses to predator odours, the role of learning in modifying the anxiety measured in the plus-maze, and the roles of cholinergic, NMDA, and dopaminergic systems. Developmental and genetic factors are considered with particular reference to the development of tests of trait anxiety. The roles of 5-HT_{1A} receptors in anxiety, depression, impulsivitity, and agonistic behaviours are discussed. Recent studies on the impacts of stress on neurotransmitter, endocrine, and immune systems and the interactions between these systems are discussed, with particular emphasis on their contributions to the development of pathologic states relevant to anxiety and depression.

Anxiety   Stress   Depression   Animal tests   Glucocorticoids   Immune function

WE all believe that anxiety, stress, and depression are interrelated. This idea is not new, but we have moved a long way from the early suggestion that, because benzodiazepines reduce both anxiety and the corticosterone stress response, the latter could be used to measure the former (95). The logical flaw in this argument is obvious and, in addition, not all anxiolytic drugs reduce the stress-induced rise in corticosterone concentrations (45), and diazepam does not counteract all stress-induced increases in corticosterone (68). The effects of the anxiolytic buspirone to elevate ACTH (adrenocorticotrophic hormone) and corticosterone concentrations (26,89, 106,108) finally destroyed any hope of using corticosterone concentrations to screen for anxiolytic activity. It had been suggested (84) that one of the reasons for the relatively weak anxiolytic effects of buspirone in animal tests was that they were being antagonised by anxiogenic effects resulting from activation of the hypothalamic–pituitary–adrenal (HPA) axis. In an elegant test of this hypothesis, McNaughton et al. (101) provide evidence of a greater anxiolytic effect of buspirone in rats with normal levels of corticosterone, but without the capacity to release endogenous corticosterone. As can be seen from the articles in this special issue, the field is advancing both in methodological developments relating to the measurement of anxiety and depression in animals and in studies of the interactions between anxiety, depression, neurotransmitters, and the endocrine and immune systems.

NEW DEVELOPMENTS IN ANIMAL TESTS OF ANXIETY

Response to Predator Odours

Every test situation to which we expose animals involves an element of stress, and the animal will learn about the nature of the aversive stimuli or stressors and the availability of response options. The different environmental stressors are likely to trigger different neurotransmitter pathways and different endocrine systems and, therefore, the animal's underlying neurobiological state will be different. There will also be different response limitations imposed by the test situation. The challenge is to further refine and understand the various tests in the hope that they will ultimately relate to the various anxiety disorders that are distinguished clinically. Hendrie et al. (71) suggest that the nature of the emotion evoked by exposure to novel situations arises from an apprehension relating to possible intraspecific encounters and, thus, is different from that evoked by exposure to a predator. Few would dispute that anxiety would be the better term to describe the emotional state evoked by the uncertainty of a novel situation, and fear would be the appropriate term for the reaction to the actual presence of a predator. The work of the Blanchards (14,16) provides evidence that the behaviours exhibited in the presence of a predator relate to fear, whereas those evoked by the odour of a predator indicate anxiety. However, there is also evidence to suggest that the nature of the anxiety induced by exposure to novelty and to predator odours may differ. The changes in GABA release and uptake are in opposite directions after exposure to novelty and cat odour (50), and benzodiazepine receptor binding changes were found in the hippocampus after exposure to the novel test situation, but in the frontal cortex after cat odour (76). There are also pharmacological differences; whereas low doses of benzodiazepines have an anxiolytic effect in animal tests employing novelty, only high doses changed behaviour during exposure to cat odour (15,156). Behaviourally, it has been shown that rats showing strong or weak avoidance of cat odour did not differ

in other tests of anxiety (77). Interestingly, although on the first exposure there is a strong correlation between behavioural and corticosterone responses to cat odour, these measures dissociate with repeated exposures, with the corticosterone, but not the behavioural, response habituating (51). Hendrie et al. (71) also suggest that the reactions of animals following exposure to predator stimuli might relate more closely to pathologic anxiety than that observed during actual contact. This would be supported by the findings that whereas chlordiazepoxide had little effect on responses to the actual odour, it was very effective at reducing the increase in anxiety that was subsequently displayed in the social interaction or elevated plus-maze tests (156). There is also neurochemical evidence for different responses in the 5-HT system occurring immediately and 30 min after odour exposure (50).

Interplay of Learning and Anxiety in the Plus-Maze

The difference between responses to predator odours and those seen in other tests of anxiety is not surprising, but there is growing evidence of important distinctions among animal tests that use novelty, light level, open spaces, or elevation as the aversive stimulus. There is mathematical evidence from factor analysis studies that measures derived from different animal tests reflect distinct and independent underlying factors (9,10,40) and, hence, may be reflecting different types or aspects of anxiety. Perhaps even more striking is the evidence that measures derived from one particular test, the elevated plus-maze, load on independent anxiety factors on trials 1 and 2 (36,52). This, together with evidence for the role of learning in changing performance on trials 1 and 2 (41,131) and pharmacological evidence (41,44,132), suggests that the nature of the anxiety that is generated by the test changes radically with experience (for example, from an unconditioned fear of open spaces on trial 1 to a rapidly acquired fear of heights on trial 2). The presence of small ledges, which some researchers have added to the open arms of the maze [see (74)] seems to prevent the acquisition of fear of heights (36).

The importance of learning during trial 1 to the changed behaviour on trial 2 is illustrated by Rodgers et al. (131), who show that considerable acquisition of aversive learning takes place even during the first minute of exposure to the maze and transfers across sessions. Although administration of scopolamine prior to trial 1 has previously been shown to affect trial 2 performance (82), somewhat surprisingly posttrial administration of doses from 0.1–1.0 mg/kg did not disrupt the behavioural change on trial 2 (131). It would be of great interest to determine whether scopolamine administration posttrial 1 would affect the response to drugs on trial 2, because it is often the response to benzodiazepines that has proved most sensitive to the changing nature of the anxiety on trials 1 and 2.

Roles of Cholinergic, NMDA, and Dopaminergic Systems

Anxiogenic effects of muscarinic antagonists have been reported for trial 1 in the plus-maze (129) and in the black-white crossing test (139). Smythe et al. (139) suggest that the anxiogenic effects could be indirectly mediated by activation of the HPA (hypothalamo–pituitary–adrenal) system, leading to increases in CRH (corticotrophin releasing hormone), ACTH, and corticosterone. These are all possible candidates for anxiogenic actions (31,39,48,49,141,142). Bhatnagar et al. (11) found that intrahippocampal administration of scopolamine enhanced the rise in ACTH and corticosterone that resulted from restraint stress and suggested that the septohippocampal

cholinergic projections involved in theta wave generation were also responsible for HPA inhibition. Thus, the cholinergic system may interact with anxiety and fear at several different levels, relating to both endocrine responses and attentional processes influencing information processing. The hippocampus may be the final common path for corticosterone-sensitive behavioural effects, but this leaves the possibility of functional antagonism from actions at other sites. For example, in electrophysiological tests of hippocampal control the direct actions of anxiolytic drugs were found to be on nuclei afferent to the septohippocampal system (100). As well as being present in the peripheral nervous system, nerve growth factor (NGF) and its receptors have been identified in the CNS, mainly in the hippocampus, cortex, and olfactory bulb (94). These regions are innervated by basal forebrain cholinergic neurones for which NGF acts as a trophic factor; in addition NGF is synthesized and released by various cell types in the endocrine and immune systems. Most interestingly, NGF plasma concentrations have been shown to increase at a time of high state anxiety, just prior to the first parachute jump (2).

The sites of interaction of anxiolytic drugs with GABA and 5-HT have been extensively investigated, but the roles of other neurotransmitter pathways should not be ignored, as indicated by anxiolytic effects of NMDA receptor antagonists (32,118). At least two neuroanatomical sites for this anxiolytic action have been identified: the periaqueductal grey (69), and the hippocampus (121). It has been known for some time that low doses of neuroleptics can have clinical antianxiety effects (125), and it was originally thought that the anxiolytic effects of buspirone were due to its dopaminergic properties (143). There is considerable evidence that acute exposure to a range of stressors increases extracellular dopamine in mesocorticolimbic areas (29), and it has been suggested that the changes in dopamine activity may relate to the coping attempts made by the animal (19). More recently, with the identification of dopaminergic receptor subtypes and the development of subreceptor specific ligands it has been possible to investigate in more detail the role of the dopaminergic system. The evidence so far would suggest little involvement of D_1 receptors in mediating changes in anxiety, but a possible role for D_2 or D_3 receptors has been demonstrated in the plus-maze (130), and the D_2 receptor agonist, piribedil, has anxiolytic effects in the social interaction test (38).

Developmental and Genetic Factors

The contributions discussed so far relate primarily to furthering our understanding of state anxiety. However, there is a growing realization of the importance of incorporating developmental and genetic factors to develop animal models of trait anxiety.

Although day 7 male and female chicks did not differ in their immediate behavioural or neurochemical responses to cat odour (a test situation evoking state anxiety), the marked sex differences in the GABA and 5-HT systems of day 7 and day 10 chicks suggested that this might form a basis for differences in their longer term responses to stress or their style of coping with repeated stress (54). It is not surprising that different types of fear response develop at different times, and an important question is the age at which an animal can show long-term modifications of such responses. In the first week of age, although rats show within-session habituation of orienting responses to an air-puff they show no long-term habituation (46), but at days 16–18 long-term habituation of explor-

atory head dipping (37) and retention of conditioned freezing responses develop, whereas long-term habituation of acoustic startle responses was found only at day 30 (119). There was little evidence of the development of social bonding in rats pair housed since weaning and then tested at day 34 after a 24-h period of separation. There were higher levels of social investigation and rough and tumble play when they were given an unfamiliar partner, than when they were reunited with their cage mate (24). This is the same pattern seen in adult rats after a period of 5 days of isolation (47). Interestingly, although both periods of social interaction testing in a novel arena raised corticosterone concentrations, there was no difference with the familiarity of the partner, again suggesting that investigation of an unfamiliar conspecific is not necessarily stressful.

Rex et al. (124) provide evidence for strong genetic and early environment effects on levels of anxiety in the rat. The genetic selection of lines of quail (for long or short tonic immobility) and mice (for low or high levels of aggression) resulted in differences in benzodiazepine receptor binding and behavioural measures of anxiety (78,79,138). In both cases, the response selection resulted in animals that could be distinguished in terms of showing active or passive coping styles to stress.

<div align="center">ROLES OF 5-HT$_{1A}$ RECEPTORS</div>

Anxiolytic Effects

The possible importance of 5-HT$_{1A}$ receptors in anxiety was raised by evidence that the clinically effective anxiolytic, buspirone, had 5-HT$_{1A}$ receptor agonist/partial agonist properties (110,146). 5-HT$_{1A}$ receptors occur both presynaptically on soma or dendrites of the raphé nuclei and postsynaptically in their projection areas. Although it is still not known for certain, there is now considerable evidence that the anxiolytic action of 5-HT$_{1A}$ receptor agonists arise primarily from their actions on the somatodendritic receptors leading to reduced 5-HT release in the terminal areas (80,136). In support of this, administration of the 5-HT$_{1A}$ receptor agonist, 8-OH-DPAT to the dorsal raphé nucleus has anxiolytic effects in the social interaction test (72,75) and in foot shock-induced vocalisations (133). However, the dorsal raphé nucleus does not play a role in all animal tests, and it was without effect on trial 1 in the plus-maze, although it did have anxiolytic effects on trial 2 (42). Thus, the role of the dorsal raphé nucleus was changed by experience with the plus-maze, which would be in agreement with the suggestion (66) that it plays a more important role in conditioned than in unconditioned fear and with the lack of involvement of the dorsal raphé nucleus in the black/white crossing test (21). However, this cannot be the complete explanation, because it is difficult to see the role of conditioning in the social interaction test and, furthermore, the application of 5-HT$_{1A}$ receptor agonists to the dorsal raphé nucleus did not have anxiolytic effects in conditioned suppression of drinking (20) or in the behavioural inhibition resulting from omission of reward (53). The role of postsynaptic 5-HT$_{1A}$ receptors in the mediation of anxiety is dependent both on the particular anatomical area and, possibly, on the particular animal test. No effect of 8-OH-DPAT has been found in either the social interaction test or in trials 1 or 2 in the plus-maze after direct application to the ventral hippocampus (42,75), whereas low doses have anxiogenic effects in the social interaction test after application to the dorsal hippocampus (3) and in the Geller-Seifter conflict test after application to the basolateral amygdala (73). High doses of 5-HT$_{1A}$ recep-

tor agonists have been reported to have anxiolytic effects after direct administration to the dorsal hippocampus, but these effects are probably due to drug diffusion to other areas (86).

Antidepressant Effects

Although there is increasing evidence that increased postsynaptic 5-HT activity is associated with an antidepressant action (4), once again this simple situation does not apply to all animal tests and for all brain areas (66). In the forced swimming test 8-OH-DPAT produced antidepressant-like effects after both dorsal raphé and septal administration (134). In an interesting series of experiments Maier et al. (103) showed that administration of 8-OH-DPAT to the dorsal raphé nucleus blocked both the potentiation of fear conditioning and the escape deficit that follows exposure to inescapable shock, regardless of whether it was administered before the inescapable shock or before the later testing. In general, anxiolytic drugs are active in this paradigm when administered prior to the inescapable shock, whereas antidepressant drugs are effective when administered prior to the subsequent testing. It would, therefore, seem that 8-OH-DPAT administration to the dorsal raphé nucleus has both anxiolytic and antidepressant effects in animal tests, and, indeed, lesions of this nucleus have also been found to have anxiolytic (43) and antidepressant effects (102). Further evidence for a presynaptic site for an an antidepressant effect comes from the effects in the forced swim test of 8-OH-DPAT administered to the DRN (22).

The role of presynaptic 5-HT$_{1A}$ receptors on the effects of acute and chronic fluoxetine on 5-HT concentrations in the frontal cortex were examined by Invernizzi et al. (81). The increase in 5-HT concentrations, measured by in vivo microdialysis, were increased by coadministration of a 5-HT$_{1A}$ receptor antagonist, which is further evidence that the enhanced antidepressant effect found clinically after coadministration of a 5-HT$_{1A}$ receptor antagonist with antidepressant medication (4) might relate to the increased postsynaptic 5-HT availability. Chronic administration of fluoxetine resulted in desensitization of the presynaptic 5-HT$_{1A}$ receptors and, therefore, the effects of endogenous 5-HT acting at this site to reduce 5-HT release would be attenuated (81). This could be one mechanism underlying the enhanced antidepressant action found after chronic treatment.

Impulsivity, Agonistic, and Sexual Behaviour

5-HT$_{1A}$ receptors play a role in controlling many different behaviours, and the extent to which these are independent of, or relate to, roles in anxiety and depression is still not clear. Numerous studies have demonstrated that animals are sensitive to delay of reward, and tests have been developed that offer a choice between an immediate, but small, reward or a delayed larger reward. The ability to tolerate a delay in reward is considered as an index of impulsivity (98). In a T-maze test using discrete trials, benzodiazepines reduced an animal's ability to wait for a reward (144), as did 5-HT$_{1A}$ receptor agonists (145), whereas serotonin uptake inhibitors increased the choice of the larger, but delayed, reward (145). However, in an analogous operant paradigm involving discrete trials and a two-lever choice, ability to wait for the larger reward was unaffected by 5-HT$_{1A}$ full or partial agonists, by benzodiazepines, or by 5-HT uptake inhibitors (23). There is no obvious explanation for the profoundly different sensitivities of the T-maze and operant paradigms, but once again, these results demonstrate that apparently comparable tests may, in fact, be

measuring different aspects of behaviour or at least may vary greatly in drug sensitivity.

In an extensive series of studies Krsiak and colleagues (92,93) found that benzodiazepines had the specific effect of reducing defensive aggression and escape in timid male mice, and suggested that this reflected an anxiolytic action. A similar pattern of results has been reported following administration of 5-HT$_{1A}$ receptor agonists (126). It is, therefore, somewhat surprising that low doses of the specific 5-HT$_{1A}$ receptor antagonist, (+)-WAY-100135 increased offensive aggression in resident male mice confronted with an intruder in their home cage (8). Anxiolytic effects of this compound have been reported in the elevated plus-maze (128) and black/white crossing test (12). The anxiolytic effects of a 5-HT$_{1A}$ receptor antagonist are somewhat surprising, but could be explained if a high 5-HT tone were engendered by the test situation and postsynaptic receptor antagonism predominated. However, this explanation can only be directly tested by examining the behavioural consequences in the tests concerned following administration of the drug to specific brain areas. The differing results in the social interaction and elevated plus-maze tests of administration of 8-OH-DPAT to the dorsal raphé nucleus (42,75) provide good evidence that different test situations lead to different patterns and levels of activity in the 5-HT pathways. Low doses of 8-OH-DPAT increased the success of subordinate rats in a social competition for a favoured food (155), in a similar way to that seen with anxiolytics acting at the benzodiazepine receptor (63,87). The aggression shown by subordinate rats in a social competition test is usually considered to be defensive (126) and, thus, this action of 8-OH-DPAT would be compatible with an anxiolytic action at presynaptic receptors. However, the chronic social stress leading to subordination has also been considered as an animal model of depression, and chronic treatment with antidepressants enhances the relative rank of subordinates (105). As with the consequences of inescapable shock, there seem to be both elements of anxiety and depression in this test situation.

Although most research on aggression has focussed on males, lactating females show high levels of aggression towards both male and female intruders. The patterns of attack are different, according to the sex of the intruder (113). Attacks on male intruders are directed at vulnerable areas of the body, such as the head, ventral surface, and inguinal area, and are accompanied by other behavioural measures of fear; it is thought that this type of aggression is defensive in nature, aimed at protection of the litter. In contrast, only offensive aggression is directed at female intruders and is accompanied by increased social investigation; it is considered that in this case the function of the aggression is to establish a social hierarchy. Fluprazine, a mixed 5-HT$_{1A/1B}$ receptor agonist reduced attacks on female, but not male intruders (114), whereas more specific 5-HT$_{1A}$ agonists caused a nonspecific reduction in attacks on male and female intruders (126). Palanza et al. (112) found that the effects of the benzodiazepine, chlordiazepoxide, depended both on the sex of the intruder and whether the lactating dam had previous experience of attacks. In inexperienced dams, chlordiazepoxide decreased defensive attack against males, but was without effect on aggression directed at female intruders. In dams with previous experience of aggression, chlordiazepoxide increased attack against males and decreased attack against females. In both situations, chlordiazepoxide modified the form of attack against males, switching it from the usual defensive aggression to one of offensive attack. Once again, this experiment illustrates the important interaction that can occur between previous learning and the effects of an anxiolytic.

Male rats exposed prenatally to stress show demasculinisation and partial feminisation of their sexual behaviour when adult (88,150). It is possible that the effects of prenatal stress are mediated, at least in part, by endogenous opioids. To examine this, the opiate antagonist, naltrexone, was administered via the drinking water to pregnant dams during their last week of pregnancy, which includes the critical stage of brain sexual differentiation. This method of treatment was chosen to avoid confounding effects from the stress of handling and injecting the pregnant females and to provide a more constant drug level. When the male offspring were tested as adults, those treated prenatally with naltrexone showed facilitated masculine sexual behaviour and suppressed feminine receptivity (25). The mechanism mediating these effects is not known, and although prenatal modulation of the hypothalamo-pituitary-gonad axis would seem an obvious candidate, this system does not mature until puberty. Neonatal treatment with 5-HT$_{1A}$ receptor agonists resulted in a decrease in offensive aggression in adult rats that was dependent on the genetic sex of the animal, but was independent of the presence of testosterone, and also a decrease in defensive aggression that was dependent on the presence of testosterone (1). There were no changes in male or female behaviour in tests of exploration, anxiety, or sexual preference or performance after neonatal treatment with 5-HT$_{1A}$ receptor agonists, but neonatal treatment with the 5-HT$_{2A/2C}$ receptor antagonist ritanserin in female, but not male, rats did result in an anxiolytic effect in adulthood (64). These findings of sex differences in response to neonatal manipulations of the 5-HT system could provide important clues for the neurobiological mechanisms that could mediate the differences in male : female incidence of anxiety and depressive disorders.

IMPACTS OF STRESS

Neurotransmitter Pathways

The literature is replete with reviews on the effects of various stressors on neurotransmitters, with most attention on GABA, 5-HT, noradrenaline, and dopamine. The importance of the GABA-benzodiazepine system in response to acute stressors is well established and, more recently, attention has turned to the interactions of neurosteroids with this receptor complex. Of the many neuroactive steroids the 3alpha-hydroxy-5alpha reduced derivatives of progesterone and deoxycorticosterone (allopregnanolone and allotetrahydrodeoxycorticosterone) are the most potent and efficacious positive modulators of GABA$_A$ receptors (117,122). These compounds also show anxiolytic properties in animal tests (13). The cerebral cortical levels of neurosteroids are altered by several stressors, but the pattern of changes depends on the particular stressor, possibly through differential activation of different neurotransmitter pathways. Barbaccia et al. (7) found that acute foot shock increased the cortical concentrations of pregnenolone, progesterone, and allotetrahydrodeoxycorticosterone, peaking 10-30 min poststress and returning to baseline after 2 h, as well as raising plasma corticosterone concentrations. These increases were antagonised by the anxiolytic, abecarnil, and mimicked by the anxiogenic, FG 7142, suggesting a functional relationship between the neurosteroids and the GABA-benzodiazepine complex.

Exposure to stress has been reported to result in subsequent increases in anxiety in other test situations. Once again, how-

ever, there is evidence that the nature of the anxiogenic response is not identical following all stressors. Thus, for example, while exposure to inescapable shock or to the odour of a cat both resulted in increased anxiety in the social interaction test (137,156), only the latter had an anxiogenic effect in the plus-maze (67,156). This is not because the plus-maze is insensitive to the anxiogenic effects of stressors, because changes have been detected following exposure to social defeat (70,127) and swim stress (17). While the reason for these differences is not known at present, one possibility is that the different stressors activate different neurotransmitter pathways, and that the two animal tests are differentially sensitive to modulation by different neurotransmitters [see (83)]. Stanford (140) discusses the importance of whether stressors involve somatosensory discomfort (e.g., inescapable shock, forced swimming, immobilization), as is typical in animal tests of depression, or exposure to novelty or signals of threat, as is typical in animal tests of anxiety. She argues that the neurochemical and behavioural changes induced by stress depend on the quality and intensity of the stress as well as the subject's history. Different aspects of the monoaminergic system respond to different aspects of a stressful situation; thus, the number of novel features in a stressful situation seems to determine the increase in cortical noradrenaline release, whereas changes in β-receptor density relate more to the intensity of the stressor (140). Long-lasting changes following stress are of particular importance to the development of pathologic responses and, thus, it is interesting that long-lasting changes in $5\text{-}HT_{2A}$ receptors were found after a single exposure to stress (140).

Fuchs et al. (60) describe a promising new animal model of depression, based on chronic psychosocial stress. In a similar manner to primates (148), subordinate tree shrews living in visual and olfactory contact with a dominant male shrew show dramatic behavioural, physiological, and neuroendocrinological changes. Subordinates withdraw from the sight of the dominant male, reduce locomotor activity, drop in body weight, cease self-grooming, and show early waking and a disrupted circadian rhythm (5,61). The endocrine changes include increased concentrations of adrenal cortical and medullary hormones (57,59) and reduced gonadal activity (149). They also demonstrate a downregulation of hippocampal glucocorticoid and CRH receptors (58,85). Both $5\text{-}HT_{1A}$ and α_2-adrenoceptors are decreased (55,56). Chronic (4 weeks) treatment with clomipramine (a selective 5-HT uptake inhibitor, but with an active metabolite that inhibits the uptake of noradrenaline) was able to reverse the already established behavioural and endocrine changes of subordinate shrews (60). The chronic mild stress model of depression is primarily based on the observation that rats subjected to a variety of stressors for a prolonged time show a marked reduction in their responsiveness to rewarding stimuli (152). Gorka et al. (65) have demonstrated that rats exposed to 4 weeks of stressors have abnormalities in the period, phase, and amplitude of their circadian rhythms, mimicking an important feature of clinical depression. Certainly a wide range of antidepressants have been found to restore abnormal circadian rhythms (151,153,154).

Recent imaging studies have highlighted the possible importance of the D_2 dopamine receptors in depression (28), and there have been clinical reports of antidepressant activity of the D_2 dopamine agonist, piribedil (107,120). D_2 dopamine agonists have also been found to be effective in the learned helplessness and forced swim animal tests of depression (33,62). Both dexamphetamine and the pure dopamine uptake

inhibitor, GBR 12783, elicited locomotor stimulation and increased swimming in the behavioural despair test in mice. The locomotor stimulant effects were antagonised by D_1 receptor antagonists, which were without effect in the swim test, whereas the D_2 receptor antagonist, haloperidol, antagonised the effects of GBR 12783 in the forced swim test, but was without effect on the locomotor stimulation (147).

In contrast to the role proposed for the dorsal raphé nucleus in state anxiety, it has been proposed that the median raphé nucleus is more concerned in coping with chronic, unavoidable stress. Interestingly, electrolytic lesions of this nucleus reduce the immune response and increase the incidence of gastric ulcers (66).

Neuroendocrine and Immune Systems

Benzodiazepines are usually considered to have antistress effects and, indeed, they reduce stress-induced increases in corticosterone secretion (39). However, not all their actions are in this direction, and diazepam and nicotine both increased Fos-like immunostaining (an early marker of neuronal activation) in stress-related brain areas (135). The dose of diazepam that was used in this study is sufficient to increase plasma corticosterone concentrations through a direct action on the peripheral benzodiazepine receptors on the adrenal cortex to stimulate steroidogenesis and, thus, an indirect role of the HPA axis is possible. This direct effect of diazepam on basal corticosterone concentrations can also be seen in the study by Groenink et al. (68), and, again, could explain its inability to counteract the stress-induced rise in corticosterone, whereas it did reduce the stress-induced rise in body temperature. In a carefully conducted study, Ebenezer et al. (35) provided evidence that questions the roles of CCK_B receptors in feeding and in stress responses. A novel tetrapeptide derived from the C-terminal sequence of CCK_8, which has the greatest potency and highest affinity of the available CCK_B agonists, was without effect on operant feeding of food-deprived pigs. Although there was a significant, but brief, rise in plasma cortisol concentrations following intravenous administration, there was no change following intracerebroventricular (ICV) injection. It was, therefore, suggested that the cortisol rise might be peripherally mediated, for example in response to an increased acid secretion in the gut. Previous studies also found that ICV pentagastrin did not affect feeding in pigs (115) or sheep (34) or raise plasma cortisol concentrations. These results suggest that caution must be exercised in assuming that the effects of CCK_{8S} on feeding and cortisol (116) are necessarily due to an action on CCK_B receptors.

Corticosterone has high affinity for mineralocorticoid and a lower affinity for glucocorticoid receptors, which occur in high densities in the amygdala, septum, and hippocampus. Thus, low circulating levels of the steroid will occupy only mineralocorticoid receptors, whereas after stress-induced increases in secretion, both types of receptor will be occupied. Korte et al. (90) found that ICV administration of a specific mineralocorticoid antagonist had anxiolytic effects in an elevated plus-maze, whereas the glucocorticoid antagonist had effects only when anxiety had been enhanced by conditioning. In the plus-maze, combined administration of both antagonists was without effect. In contrast, only the simultaneous blockade of both receptor types produced behavioural changes in the defensive burying and potentiated startle tests of anxiety (91). In particular, the behavioural changes reflected a shift from active to passive coping strategies, with a

small decrease in active burying being accompanied by a large increase in immobility. It would be interesting to see whether ethological measures of behaviour in the plus-maze (36,127) would identify any similar shift in the type of behaviour displayed. Active coping strategies are accompanied by an increase in plasma noradrenaline and tachycardia, indicating an increase in sympathetic nervous activity; passive coping is accompanied by increases in plasma noradrenaline, adrenaline, and ACTH (27). The patterns of response both available to an animal, and displayed by individuals, in different test situations will, therefore, determine both the neuroendocrine and neurotransmitter activity, and, hence, influence the animal's sensitivity to different drugs.

The importance of sex differences in the aetiology of anxiety and depression is only just beginning to be addressed in preclinical research. It is, therefore, of great interest that there is an important sexual dimorphism in the immunological response. The role of steroids is preeminent in the development of this dimorphism; thus, gonadectomy in developing rats will abolish the sexual dimorphism, whereas in adulthood gonadectomy only attenuates the difference (97).

Overnight restraint stress of mice results in a suppression of various aspects of the immune response (Con-A-driven lymphocyte proliferation, plaque-forming cell response to sheep red blood cells and NK-activity in the spleen) but increased phagocytic activity (104). The effects on these effects (except those on NK cell activity) of restraint stress were reduced by pretreatment with methionine-enkephalin, but this compound had effects on the immune system similar to those of stress. Pretreatment with the opioid antagonist, naloxone, reversed the stress-induced increase in phagocytosis and the decrease of T-cell proliferation. It was suggested (104) that the stress-induced alterations were mediated by at least two mechanisms: activation of the HPA axis, which affected T/B-lymphocytes and secretion of endogenous opioid peptides, which affected phagocytes. The endogenous opioids could act in two ways, directly on immunocytes, or indirectly, by in turn activating the HPA axis.

Buckingham et al. (18) provide a substantial and detailed review of the manner in which challenges to the immune system (from infection or inflammation) initiate diverse changes in endocrine function, the most overt of which is activation of the HPA axis. The roles and interactions of the interleukins, eicosanoids, and glucocorticoids are discussed. In some cases, mediators act directly on the HPA system; others act locally at the site of the immune/inflammatory lesion and others cause changes (e.g., hypoglycaemia or hypotension) which in turn are stressors activating the HPA system. There is extensive clinical evidence for changes in the immune system during depression, mediated by glucocorticoids, cytokines, and CRH, and the roles of stress and the immune system in the etiology of anxiety and depression are briefly reviewed by Leonard and Song (96). An exciting recent development is the potential role of CRH binding protein (99) and its putative peripheral ligands (i.e., the elusive tissue CRH) in immune/inflammatory disease. These peptides could underlie many of the extrahypothalamic actions of CRH, and it would be of great interest to know whether they could mediate changes in anxiety and depression (30,109) and the increased anxiety that occurs on withdrawal from chronic alcohol (6,123) or benzodiazepine treatment (111).

CONCLUSIONS

The articles presented in this volume provide an excellent illustration of the diversity of research approaches to the very important clinical problems posed by the anxiety and depressive disorders. Only by fully considering genetic and early developmental aspects and bearing in mind at all stages the importance of sex differences in response to stressors will we arrive at a better understanding of the complex interactions that ultimately lead to the development of a pathologic response. It is also vital that we continue to make progress in our development and understanding of animal models of anxiety and depression, because we will always need behavioural tests in this area. The literature already has sufficient examples of dissociations between behavioural and endocrine responses for us to realize that we cannot treat the various reactions to stressors as interchangeable measures of a single underlying pathology.

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