Symposium

Brain neuropeptide Y and corticotropin-releasing hormone in mediating stress and anxiety

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Abstract

Neuropeptides such as neuropeptide Y (NPY) and corticotropin-releasing hormone (CRH) have been implicated not only in acute regulation of stress/anxiety-related behaviors, but adaptations and changes in these neuropeptide systems may also participate in the regulation of behavior and endocrine responses during chronic stress. NPY is an endogenous anxiolytic neuropeptide, while CRH has anxiogenic properties upon central administration. Changes in these neuropeptide systems may contribute to disease states and give us indications for putative treatment targets for stress/anxiety disorders as well as alcohol/drug dependence. In this review, we briefly present these two systems and review their involvement in mediating the responses to acute and chronic stressors, as well as their possible roles in the development and progression of stress/anxiety disorders. We suggest that neuropeptides may be attractive in treatment development for stress/anxiety disorders, as well as for alcohol/drug dependence, based on their specificity and activity following exposure to external challenges, i.e. stressors, and their differential adaptations during transition from an acute to a chronic stress exposure state.

Keywords: neuropeptide Y, corticotropin-releasing hormone, anxiety, stress

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Background

Under acute conditions, stress invokes a response that may be beneficial for survival. However, when activated chronically, stress responses can cause damage and be the basis for, as well as accelerate, disease conditions.[1–4] Stress as a term refers to processes involving perception, appraisal and response to threatening, challenging and/or possibly harmful stimuli. There exists a division of stressful stimuli into emotional, or psychological, stimuli and physiological stimuli. Examples of stimuli that are considered emotional/psychological include social interactions/conflicts, inescapable situations, death of a loved one and so forth. Physiological stimuli can be hypo/hyperthermia, an immune challenge, starvation and also drug withdrawal.

The internal stress response can be related by the term allostasis and refers to the process of re-establishing homeostasis in response to a challenge.[5,6] Homeostasis refers to consistency of internal parameters within a normal range, while allostasis describes the body's way to keep stable outside the normal range by changing internal systems to match external demands.[5,6] Allostasis links the brain with the endocrine system as well as our immune system to coordinate appropriate responses to an (external) stressor.[7]

In addition to being intrinsically harmful, (chronic) stress has also been shown to increase vulnerability to addiction. Drug intake and withdrawal impose a stress on internal systems leading to a disruption of the homeostatic state. Furthermore, repeated exposure to and withdrawal from drug use leads to increased sensitivity to stress and an increased behavioral stress response.[8] In addition, stress-induced relapse is a model frequently used in preclinical settings and involves exposure to a stressor (foot-shock for example), which then leads to the reinstatement of a previously extinguished behavior, drug use (for example, see reference[9]).

The terms stress, fear and anxiety are at times used interchangeably; however, they are considered different in the area of anxiety research. As stated above, stress refers to a real or inferred threat affecting numerous systems in the body leading to a response, and may be defined as a force changing a (previously) static condition. Fear may be the result of the stress response itself and is associated with a specific event or stressor. Anxiety cannot be related to a specific event, but may be due to chronic stress or fear. It is a more general response and may be indicative of changes on both neuronal and system-wide levels. Anxious individuals emotionally anticipate an aversive situation.

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Approximately one in four individuals will experience an anxiety-related disorder during their lifetime.[10] This together with well-known issues such as side-effects and limited efficacy for existing anxiolytics makes the need for new treatments urgent. Neuropeptide systems may offer opportunities for such treatment development. Neuropeptides have been suggested to only be released if the firing of a neuron exceeds a certain level, i.e. frequency-dependent release.[11] Furthermore, neuropeptides, when compared with classical neurotransmitters, can act over longer distances and may thus be used to recruit large neuronal populations to produce a concerted physiological effect.[12] Additionally, the accessibility of neuropeptides is higher due to limited reuptake mechanisms as compared with classical neurotransmitters.

In this brief review, we discuss the involvement of two neuropeptide systems: corticotropin-releasing hormone (CRH) and neuropeptide Y NPY) in stress-related behavior and anxiety, and the putative opposing roles of these peptides in the behavioral responses to stress.

Corticotropin-releasing hormone

CRH is a 41-amino-acid neuropeptide that is released by the hypothalamus upon exposure to a stressful stimulus. It mediates autonomic,[13,14] neuroendocrine[14] and behavioral responses to stress.[15,16] CRH activates the hypothalamic-pituitary-adrenal axis (HPA) by stimulating release of adrenocorticotropic hormone (ACTH) from the pituitary, which in turn stimulates release of cortisol/corticosterone (human/rodent) from the adrenal cortex. The stress-induced activation of the HPA axis is dependent on CRH.[17] In addition to HPA axis activation, CRH participates in extrahypothalamic behavioral responses to stress.[18] The neuroanatomical substrates for CRH, in addition to the hypothalamus, include, among others, the amygdala, the bed nucleus of the stria terminalis and the locus coeruleus.[19–21]

CRH is a member of a peptide family including Urocortin, Urocortin II and Urocortin III.[22,23] The effects of these peptides are mediated via two G-protein (G_s)-coupled receptors: CRH-R1 and CRH-R2.[24] Agonist activation of these receptors leads to increased intracellular concentrations of cyclic AMP (cAMP). CRH-R1 is the primary receptor for CRH and participates in mediation of the behavioral stress response.[25,26]

Central administration of CRH is anxiogenic in several animal behavioral tests such as the novel open-field,[27] acoustic startle[28] and operant conflict tests.[29] Whether the centrally mediated, anxiogenic behavioral effects of CRH are dependent on the HPA axis, or completely independent thereof requires further discussion. Lesions of the central nucleus of the amygdala, but not the hypothalamic paraventricular nucleus (PVN), disrupt CRH-potentiated fear responses,[30] and peripheral blockade of ACTH/glucocorticoids does not affect the behavioral response.[31] Furthermore, central administration of CRH in dexamethasone-treated rats still elicits an anxiogenic effect.[32] However, adrenalectomy abolishes the locomotor activity-inducing effects of central CRH administration.[33] In preclinical studies, stress increases CRH release in the amygdala[34,35] as well as CRH mRNA expression.[26,36] Furthermore, in non-human primate models of early adverse events chronic elevation of cerebrospinal fluid (CSF) CRH is seen,[37] and patients with post-traumatic stress disorder (PTSD) have significant elevation of CSF CRH,[38] indicating significant activation of the extrahypothalamic CRH system in these conditions.

Given the above studies, administration of CRH-R1 receptor antagonists may offer an effective treatment for stress-induced anxiety disorders. Indeed, treatment with CRH-R1 receptor antagonists does result in decreased levels of experimental anxiety in rats with a 'high anxiety' phenotype,[39] in mice following exposure to a chronic mild stress paradigm,[40] as well as in human subjects diagnosed with major depression or PTSD.[41–43] Furthermore, a CRH-R1 antagonist was effective in blocking alcohol withdrawal-induced anxiety on the elevated plus-maze as well as stress-induced reinstatement of alcohol seeking.[44] Similar to other potent stressors, acute alcohol withdrawal induces anxiety-like responses that are correlated with increased CRH levels in the central nucleus of the amygdala and in the bed nucleus of the stria terminalis,[45] sites mediating the behavioral responses to stress. Accordingly, anxiogenic effects of alcohol withdrawal are reversed by CRH-R1 antagonism.[44,46] It should be noted that limited to no effect of CRH-R1 antagonists upon administration to naïve/unstressed animals was seen, suggesting that the CRH system may have limited tone under baseline (non-challenged) conditions.

Neuropeptide Y

NPY is a 36-amino-acid peptide widely expressed in the mammalian nervous system, with high levels in brain regions such as the hypothalamus, in particular the arcuate and the paraventricular nuclei, the hippocampal formation, the amygdala and septum. Following its isolation,[47,48] NPY has been extensively studied with particular attention to anxiety/stress-related behavior and feeding.

To date, all NPY receptors cloned belong to the superfamily of G-protein-coupled receptors, but differ in their ligand affinity profiles. NPY receptors are coupled via $G_{i/o}$ proteins to several downstream signaling pathways, including inhibition of adenylyl cyclase, activation of mitogen-activated protein kinase, regulation of intracellular calcium (Ca^{2+}) concentrations and activation of G-protein-coupled, inwardly rectifying potassium (K^+) channels. Currently, there are four subtypes of NPY receptors known to mediate biological responses: Y1, Y2, Y4 and Y5. The predominantly postsynaptic Y1 receptor requires the intact NPY sequence for recognition and activation, and has been proposed as the subtype mediating antianxiety actions of NPY.[49] The presynaptic Y2 receptor subtype is in addition activated by C-terminal fragments of NPY, such as NPY^{13-36} and NPY^{3-36}.[50,51] The Y4 receptor has low affinity for NPY and is primarily the target for pancreatic polypeptide (PP), another member of the PP family of peptides.[52] The Y5 receptor was initially thought to be the receptor regulating NPY's effect on feeding behavior and Y5 receptor

antagonists have been evaluated for putative obesity treatment, possibly in combination with compounds aimed at other NPY receptors such as the Y1.[53,54]

Early indications of anxiolytic/sedative properties of NPY included synchronization of electroencephalographic activity,[55] the prevention of stress-induced ulcers and suppression of locomotor activity.[56,57] Central administration of exogenous NPY produces an anxiolytic phenotype in numerous animal models of experimental anxiety.[58–60] Antianxiety effects of NPY have been shown to rely in part on the activation of Y1 receptors in the amygdala.[61–63] Intracerebroventricular injections of NPY or Y1 receptor agonists, but not Y2 receptor agonists, are anxiolytic in behavioral models of experimental anxiety.[64]

In agreement with antistress effects observed following central administration of NPY, a role for endogenous NPY in the control of stress- and anxiety-related behaviors is suggested by several findings. Acute physical restraint, which promotes experimental anxiety, suppresses NPY mRNA and peptide levels within the amygdala and cortex.[65] In contrast, repeated exposure to the same stressor once daily for 10 d leads to a complete behavioral and endocrine habituation, accompanied by an upregulation of amygdala NPY expression.[66] Furthermore, chronic overexpression of NPY generates a stress-insensitive and 'anxiolytic' phenotype.[67,68] Genetic variations in human NPY expression affects stress response and emotion.[69,70] We have therefore proposed that an upregulation of NPY expression may contribute to the behavioral adaptation to stress. This extends a hypothesis that NPY may act to 'buffer' behavioral effects of stress-promoting signals.[71]

Evidence for interactions between CRH and NPY systems

Cannon (1927)[72] pioneered the concept of a specific site regulating emotional responses. Later work by Papez (1937)[73] and Klüver and Bucy (1937)[74,75] confirmed a role for, among other regions, the amygdala in emotional regulation. Studies in human subjects[76,77] as well as animals[78,79] have since shown that activation of the amygdala is crucial for the mediation of behavioral and emotional responses associated with anxiety. Lesioning of the amygdala in turn leads to decreased levels of experimental anxiety and decreased sensitivity to stress.[80,81] A role has been proposed for the amygdala to integrate inputs from the varying brain regions to produce a coherent behavioral response to fear and anxiety. In part this response is mediated by neuropeptides including, but not limited to, NPY and CRH.

NPY and CRH have indeed been demonstrated to have presence in many brain regions relevant for the behavioral and emotional response to stressful stimuli and/or fear/anxiety-producing events. In 1994, Heilig *et al.*[71] proposed opposing roles for NPY and CRH within the amygdala to maintain emotional balance following stress exposure. NPY was suggested to be released subsequently to increases in amygdalar CRH producing an opposing action on the stress system, facilitating return to a homeostatic (or allostatic) balance.

As discussed above, numerous studies have indicated anxiolytic and anxiogenic effects of NPY and CRH, respectively, upon administration into the CNS. In addition, studies have revealed overlapping biological substrates, primarily within the amygdala, for these effects. Both CRH and NPY receptors have been found within the amygdala.[82–85] The basolateral nucleus (BLA) is of particular interest since CRH, as well as Urocortin I, elicit long-term anxiety-like responses upon administration into the BLA.[86,87] However, the opposite behavioral response is seen upon BLA administration of NPY.[63] Furthermore, pretreatment with NPY into the BLA prior to administration of Urocortin blocks the anxiogenic effect of the CRH-R1/2 receptor activation on anxiety-related behavior.[12,88] This indicates a direct interaction of CRH and NPY systems within the BLA to modify anxiety-related behavior. The molecular mechanism for this interaction has been proposed to be differential regulation of cAMP levels.[89] In addition, at the level of the hypothalamus, dose-dependent increases in NPY overflow from the PVN were observed following CRH administration.[90] Further investigation into the clinical implications for these interactions is needed.

Concluding remarks

In conclusion, this review briefly discusses the importance of two neuropeptide systems, CRH and NPY, with regards to their individual and overlapping contribution to stress/anxiety-related behaviors and responses. Neuropeptide systems may offer more attractive treatment targets than the classical neurotransmitter systems due to the limited tonic activity in neuropeptide systems. Lack of tonic activity may generate treatments that have efficacy only during times of specific neuronal activity and may thus lead to state-specific treatments as well as a reduction in the appearance of side-effects.

The opposing roles of CRH and NPY in the regulation of stress/anxiety-related behaviors and responses may open the way for putative combination treatments aimed at targets within these systems.

REFERENCES

1 McEwen BS. The neurobiology of stress: from serendipity to clinical relevance. *Brain Res* 2000;**886**:172–89
2 Sapolsky RM. Physiological and pathophysiological implications of social stress in mammals. In: McEwen BS, Goodman HM, eds. *Coping with the Environment: Neural and Endocrine Mechanisms*. New York: Oxford University Press, 2001:517
3 Sapolsky RM. Endocrinology of the stress-response. In: Becker J, Breedlove S, Crews D, McCarthy M, eds. *Behavioral Endocrinology*. Cambridge, MA: MIT Press, 2002:409
4 Sapolsky RM. Stress and plasticity in the limbic system. *Neurochem Res* 2003;**28**:1735–42
5 Koob GF, Le Moal M. Drug abuse: hedonic homeostatic dysregulation. *Science* 1997;**278**:52–8
6 Koob GF, Le Moal M. Drug addiction, dysregulation of reward, and allostasis. *Neuropsychopharmacology* 2001;**24**:97–129
7 McEwen BS. Stress, adaptation, and disease. Allostasis and allostatic load. *Ann NY Acad Sci* 1998;**840**:33–44
8 Sommer WH, Rimondini R, Hansson AC, Hipskind PA, Gehlert DR, Barr CS, Heilig M. Upregulation of voluntary alcohol intake, behavioral sensitivity to stress, and amygdala Crhr1 expression following a history of dependence. *Biol Psychiatry* 2008;**63**:139–45

9 Shaham Y, Funk D, Erb S, Brown TJ, Walker CD, Stewart J. Corticotropin-releasing factor, but not corticosterone, is involved in stress-induced relapse to heroin-seeking in rats. *J Neurosci* 1997;**17**:2605–14

10 Kessler RC, McGonagle KA, Zhao S, Nelson CB, Hughes M, Eshleman S, Wittchen HU, Kendler KS. Lifetime and 12-month prevalence of DSM-III-R psychiatric disorders in the United States. Results from the National Comorbidity Survey. *Arch Gen Psychiatry* 1994;**51**:8–19

11 Hokfelt T. Neuropeptides in perspective: the last ten years. *Neuron* 1991;**7**:867–79

12 Sajdyk TJ, Shekhar A, Gehlert DR. Interactions between NPY and CRF in the amygdala to regulate emotionality. *Neuropeptides* 2004;**38**:225–34

13 Dunn AJ, Berridge CW. Physiological and behavioral responses to corticotropin-releasing factor administration: is CRF a mediator of anxiety or stress responses? *Brain Res Brain Res Rev* 1990;**15**:71–100

14 Vale W, Spiess J, Rivier C, Rivier J. Characterization of a 41-residue ovine hypothalamic peptide that stimulates secretion of corticotropin and beta-endorphin. *Science* 1981;**213**:1394–7

15 Heinrichs SC, Menzaghi F, Pich EM, Baldwin HA, Rassnick S, Britton KT, Koob GF. Anti-stress action of a corticotropin-releasing factor antagonist on behavioral reactivity to stressors of varying type and intensity. *Neuropsychopharmacology* 1994;**11**:179–86

16 Koob GF. Stress, corticotropin-releasing factor, and drug addiction. *Ann NY Acad Sci* 1999;**897**:27–45

17 Rivier CL, Plotsky PM. Mediation by corticotropin releasing factor (CRF) of adenohypophysial hormone secretion. *Annu Rev Physiol* 1986;**48**:475–94

18 Heinrichs SC, Koob GF. Corticotropin-releasing factor in brain: a role in activation, arousal, and affect regulation. *J Pharmacol Exp Ther* 2004;**311**:427–40

19 Gray TS, Bingaman EW. The amygdala: corticotropin-releasing factor, steroids, and stress. *Crit Rev Neurobiol* 1996;**10**:155–68

20 Valentino RJ, Wehby RG. Corticotropin-releasing factor: evidence for a neurotransmitter role in the locus ceruleus during hemodynamic stress. *Neuroendocrinology* 1988;**48**:674–7

21 Schulkin J, Gold PW, McEwen BS. Induction of corticotropin-releasing hormone gene expression by glucocorticoids: implication for understanding the states of fear and anxiety and allostatic load. *Psychoneuroendocrinology* 1998;**23**:219–43

22 Dautzenberg FM, Hauger RL. The CRF peptide family and their receptors: yet more partners discovered. *Trends Pharmacol Sci* 2002;**23**:71–7

23 Reul JM, Holsboer F. Corticotropin-releasing factor receptors 1 and 2 in anxiety and depression. *Curr Opin Pharmacol* 2002;**2**:23–33

24 Sanchez MM, Young LJ, Plotsky PM, Insel TR. Autoradiographic and *in situ* hybridization localization of corticotropin-releasing factor 1 and 2 receptors in nonhuman primate brain. *J Comp Neurol* 1999;**408**:365–77

25 Contarino A, Heinrichs SC, Gold LH. Understanding corticotropin releasing factor neurobiology: contributions from mutant mice. *Neuropeptides* 1999;**33**:1–12

26 Muller MB, Wurst W. Getting closer to affective disorders: the role of CRH receptor systems. *Trends Mol Med* 2004;**10**:409–15

27 Britton DR, Koob GF, Rivier J, Vale W. Intraventricular corticotropin-releasing factor enhances behavioral effects of novelty. *Life Sci* 1982;**31**:363–7

28 Swerdlow NR, Geyer MA, Vale WW, Koob GF. Corticotropin-releasing factor potentiates acoustic startle in rats: blockade by chlordiazepoxide. *Psychopharmacology (Berl)* 1986;**88**:147–52

29 Britton KT, Morgan J, Rivier J, Vale W, Koob GF. Chlordiazepoxide attenuates response suppression induced by corticotropin-releasing factor in the conflict test. *Psychopharmacology (Berl)* 1985;**86**:170–4

30 Liang KC, Melia KR, Campeau S, Falls WA, Miserendino MJ, Davis M. Lesions of the central nucleus of the amygdala, but not the paraventricular nucleus of the hypothalamus, block the excitatory effects of corticotropin-releasing factor on the acoustic startle reflex. *J Neurosci* 1992;**12**:2313–20

31 Pich EM, Heinrichs SC, Rivier C, Miczek KA, Fisher DA, Koob GF. Blockade of pituitary–adrenal axis activation induced by peripheral immunoneutralization of corticotropin-releasing factor does not affect the behavioral response to social defeat stress in rats. *Psychoneuroendocrinology* 1993;**18**:495–507

32 Britton KT, Lee G, Vale W, Rivier J, Koob GF. Corticotropin releasing factor (CRF) receptor antagonist blocks activating and 'anxiogenic' actions of CRF in the rat. *Brain Res* 1986;**369**:303–6

33 Korte SM, Eisinga W, Timmerman W, Nyakas C, Bohus B. Behavioral and cardiac responses after intracerebroventricular corticotropin-releasing hormone (CRH) administration: role of adrenal cortical hormones. *Horm Behav* 1992;**26**:375–84

34 Merali Z, McIntosh J, Kent P, Michaud D, Anisman H. Aversive and appetitive events evoke the release of corticotropin-releasing hormone and bombesin-like peptides at the central nucleus of the amygdala. *J Neurosci* 1998;**18**:4758–66

35 Merlo PE, Lorang M, Yeganeh M, Rodriguez de FF, Raber J, Koob GF, Weiss F. Increase of extracellular corticotropin-releasing factor-like immunoreactivity levels in the amygdala of awake rats during restraint stress and ethanol withdrawal as measured by microdialysis. *J Neurosci* 1995;**15**:5439–47

36 Kalin NH, Takahashi LK, Chen FL. Restraint stress increases corticotropin-releasing hormone mRNA content in the amygdala and paraventricular nucleus. *Brain Res* 1994;**656**:182–6

37 Coplan JD, Andrews MW, Rosenblum LA, Owens MJ, Friedman S, Gorman JM, Nemeroff CB. Persistent elevations of cerebrospinal fluid concentrations of corticotropin-releasing factor in adult nonhuman primates exposed to early-life stressors: implications for the pathophysiology of mood and anxiety disorders. *Proc Natl Acad Sci USA* 1996;**93**:1619–23

38 Baker DG, West SA, Nicholson WE, Ekhator NN, Kasckow JW, Hill KK, Bruce AB, Orth DN, Geracioti TD Jr. Serial CSF corticotropin-releasing hormone levels and adrenocortical activity in combat veterans with posttraumatic stress disorder. *Am J Psychiatry* 1999;**156**:585–8

39 Keck ME, Welt T, Wigger A, Renner U, Engelmann M, Holsboer F, Landgraf R. The anxiolytic effect of the CRH(1) receptor antagonist R121919 depends on innate emotionality in rats. *Eur J Neurosci* 2001;**13**:373–80

40 Ducottet C, Griebel G, Belzung C. Effects of the selective nonpeptide corticotropin-releasing factor receptor 1 antagonist antalarmin in the chronic mild stress model of depression in mice. Prog Neuropsychopharmacol *Biol Psychiatry* 2003;**27**:625–31

41 Zobel AW, Nickel T, Kunzel HE, Ackl N, Sonntag A, Ising M, Holsboer F. Effects of the high-affinity corticotropin-releasing hormone receptor 1 antagonist R121919 in major depression: the first 20 patients treated. *J Psychiatr Res* 2000;**34**:171–81

42 Adamec R, Fougere D, Risbrough V. CRF receptor blockade prevents initiation and consolidation of stress effects on affect in the predator stress model of PTSD. *Int J Neuropsychopharmacol* 2009:1–11 [Epub ahead of print]

43 Strohle A, Scheel M, Modell S, Holsboer F, Blunted. ACTH response to dexamethasone suppression-CRH stimulation in posttraumatic stress disorder. *J Psychiatr Res* 2008;**42**:1185–8

44 Gehlert DR, Cippitelli A, Thorsell A, Le AD, Hipskind PA, Hamdouchi C, Lu J, Hembre EJ, Cramer J, Song M, McKinzie D, Morin M, Ciccocioppo R, Heilig M. 3-(4-Chloro-2-morpholin-4-yl-thiazol-5-yl)-8-(1-ethylpropyl)-2,6-dimethyl-imidazo[1,2-*b*]pyridazine: a novel brain-penetrant, orally available corticotropin-releasing factor receptor 1 antagonist with efficacy in animal models of alcoholism. *J Neurosci* 2007;**27**:2718–26

45 Olive MF, Koenig HN, Nannini MA, Hodge CW. Elevated extracellular CRF levels in the bed nucleus of the stria terminalis during ethanol withdrawal and reduction by subsequent ethanol intake. *Pharmacol Biochem Behav* 2002;**72**:213–20

46 Baldwin HA, Rassnick S, Rivier J, Koob GF, Britton TK. CRF antagonist reverses the 'anxiogenic' response to ethanol withdrawal in the rat. *Psychopharmacology (Berl)* 1991;**103**:227–32

47 Tatemoto K, Carlquist M, Mutt V. Neuropeptide Y – a novel brain peptide with structural similarities to peptide YY and pancreatic polypeptide. *Nature* 1982;**296**:659–60

48 Tatemoto K. Neuropeptide Y: complete amino acid sequence of the brain peptide. *Proc Natl Acad Sci USA* 1982;**79**:5485–9

49 Heilig M, McLeod S, Brot M, Heinrichs SC, Menzaghi F, Koob GF, Britton KT. Anxiolytic-like action of neuropeptide Y: mediation by Y1 receptors in amygdala, and dissociation from food intake effects. *Neuropsychopharmacology* 1993;**8**:357–63

50 Wahlestedt C, Yanaihara N, Håkanson R. Evidence for different pre- and post-junctional receptors for neuropeptide Y and related peptides. *Regul Pept* 1986;**13**:301–7

51 Wahlestedt C, Hakanson R. Effects of neuropeptide Y (NPY) at the sympathetic neuroeffector junction. Can pre- and postjunctional receptors be distinguished? *Med Biol* 1986;**64**:85–8

52 Bard JA, Walker MW, Branchek TA, Weinshank RL. Cloning and functional expression of a human Y4 subtype receptor for pancreatic polypeptide, neuropeptide Y, and peptide YY. *J Biol Chem* 1995;**270**:26762–5

53 Schaffhauser AO, Stricker-Krongrad A, Brunner L, Cumin F, Gerald C, Whitebread S, Criscione L, Hofbauer KG. Inhibition of food intake by neuropeptide Y Y5 receptor antisense oligodeoxynucleotides. *Diabetes* 1997;**46**:1792–8

54 O'Shea D, Morgan DG, Meeran K, Edwards CM, Turton MD, Choi SJ, Heath MM, Gunn I, Taylor GM, Howard JK, Bloom CI, Small CJ, Haddo O, Ma JJ, Callinan W, Smith DM, Ghatei MA, Bloom SR. Neuropeptide Y induced feeding in the rat is mediated by a novel receptor. *Endocrinology* 1997;**138**:196–202

55 Fuxe K, Agnati LF, Harfstrand A, Zini I, Tatemoto K, Pich EM, Hokfelt T, Mutt V, Terenius L. Central administration of neuropeptide Y induces hypotension bradypnea and EEG synchronization in the rat. *Acta Physiol Scand* 1983;**118**:189–92

56 Heilig M, Murison R. Intracerebroventricular neuropeptide Y suppresses open field and home cage activity in the rat. *Regul Pept* 1987;**19**:221–31

57 Heilig M, Murison R. Intracerebroventricular neuropeptide Y protects against stress-induced gastric erosion in the rat. *Eur J Pharmacol* 1987;**137**:127–9

58 Broqua P, Wettstein JG, Rocher MN, Gauthier-Martin B, Junien JL. Behavioral effects of neuropeptide receptor agonists in the elevated plus-maze and fear-potentiated startle procedure. *Behav Pharmacol* 1995;**6**:215–22

59 Heilig M, Soderpalm B, Engel JA, Widerlov E. Centrally administered neuropeptide Y (NPY) produces anxiolytic-like effects in animal anxiety models. *Psychopharmacology (Berl)* 1989;**98**:524–9

60 Heilig M, McLeod S, Koob GF, Britton KT. Anxiolytic-like effect of neuropeptide Y (NPY), but not other peptides in an operant conflict test. *Regul Pept* 1992;**41**:61–9

61 Wahlestedt C, Pich EM, Koob GF, Yee F, Heilig M. Modulation of anxiety and neuropeptide Y–Y1 receptors by antisense oligodeoxynucleotides. *Science* 1993;**259**:528–31

62 Heilig M. Antisense inhibition of neuropeptide Y (NPY) –Y1 receptor expression blocks the anxiolytic like action of NPY in amygdala and paradoxically increases feeding. *Regul Pept* 1995;**59**:201–5

63 Sajdyk TJ, Vandergriff MG, Gehlert DR. Amygdalar neuropeptide Y Y-1 receptors mediate the anxiolytic-like actions of neuropeptide Y in the social interaction test. *Eur J Pharmacol* 1999;**368**:143–7

64 Britton KT, Southerland S, Van Uden E, Kirby D, Rivier J, Koob GF. Anxiolytic activity of NPY receptor agonists in the conflict test. *Psychopharmacology (Berl)* 1997;**132**:6–13

65 Thorsell A, Svensson P, Wiklund L, Sommer W, Ekman R, Heilig M. Suppressed neuropeptide Y (NPY) mRNA in rat amygdala following restraint stress. *Regul Pept* 1998;**75–6**:247–54

66 Thorsell A, Carlsson K, Ekman R, Heilig M. Behavioral and endocrine adaptation, and up-regulation of NPY expression in rat amygdala following repeated restraint stress. *Neuroreport* 1999;**10**:3003–7

67 Thorsell A, Michalkiewicz M, Dumont Y, Quirion R, Caberlotto L, Rimondini R, Mathe AA, Heilig M. Behavioral insensitivity to restraint stress, absent fear suppression of behavior and impaired spatial learning in transgenic rats with hippocampal neuropeptide Y overexpression. *Proc Natl Acad Sci USA* 2000;**97**:12852–7

68 Thorsell A, Repunte-Canonigo V, O'Dell LE, Chen SA, King AR, Lekic D, Koob GF, Sanna PP. Viral vector-induced amygdala NPY overexpression reverses increased alcohol intake caused by repeated deprivations in Wistar rats. *Brain* 2007;**130**:1330–7

69 Zhou Z, Zhu G, Hariri AR, Enoch MA, Scott D, Sinha R, Virkkunen M, Mash DC, Lipsky RH, Hu XZ, Hodgkinson CA, Xu K, Buzas B, Yuan Q, Shen PH, Ferrel RE, Manuck SB, Brown SM, Hauger RL, Stohler CS, Zubieta JK, Goldman D. Genetic variation in human NPY expression affects stress response and emotion. *Nature* 2008;**452**:997–1001

70 Heilig M, Zachrisson O, Thorsell A, Ehnvall A, Mottagui-Tabar S, Sjogren M, Asberg M, Ekman R, Wahlestedt C, Agren H. Decreased cerebrospinal fluid neuropeptide Y (NPY) in patients with treatment refractory unipolar major depression: preliminary evidence for association with preproNPY gene polymorphism. *J Psychiatr Res* 2004;**38**:113–21

71 Heilig M, Koob GF, Ekman R, Britton KT. Corticotropin-releasing factor and neuropeptide Y: role in emotional integration. *Trends Neurosci* 1994;**17**:80–5

72 Cannon WB. The James-Lange theory of emotion: a critical examination and an alternative theory. *Am J Psychol* 1927;**39**:106–24

73 Papez JW. A proposed mechanism of emotion. *Arch Neurol Psychiatry* 1937;**79**:217–24

74 Klüver H, Bucy PC. 'Psychic blindness' and other symptoms following bilateral temporal lobectomy in rhesus monkeys. *Am J Physiol* 1937;**119**:352–3

75 Klüver H, Bucy PC. Preliminary analysis of functions of the temporal lobes in monkeys. *Arch Neurol Psychiatry* 1939;**42**:979–1000

76 Brambilla P, Barale F, Caverzasi E, Soares JC. Anatomical MRI findings in mood and anxiety disorders. *Epidemiol Psychiatr Soc* 2002;**11**:88–99

77 Davidson RJ. The functional neuroanatomy of affective style. In: Lane RD, Nadel L, eds. In *Cognitive Neuroscience of Emotion*. New York: Oxford University Press, 2000:371

78 Davis M. The role of the amygdala in fear-potentiated startle: implications for animal models of anxiety. *Trends Pharmacol Sci* 1992;**13**: 35–41

79 Sanders SK, Shekhar A. Regulation of anxiety by GABAA receptors in the rat amygdala. *Pharmacol Biochem Behav* 1995;**52**:701–6

80 Moller C, Wiklund L, Sommer W, Thorsell A, Heilig M. Decreased experimental anxiety and voluntary ethanol consumption in rats following central but not basolateral amygdala lesions. *Brain Res* 1997;**760**:94–101

81 Amaral DG. The primate amygdala and the neurobiology of social behavior: implications for understanding social anxiety. *Biol Psychiatry* 2002;**51**:11–17

82 Lovenberg TW, Chalmers DT, Liu C, De Souza EB. CRF2 alpha and CRF2 beta receptor mRNAs are differentially distributed between the rat central nervous system and peripheral tissues. *Endocrinology* 1995;**136**:4139–42

83 Parker RM, Herzog H. Regional distribution of Y-receptor subtype mRNAs in rat brain. *Eur J Neurosci* 1999;**11**:1431–48

84 Van PK, Viau V, Bittencourt JC, Chan RK, Li HY, Arias C, Prins GS, Perrin M, Vale W, Sawchenko PE. Distribution of mRNAs encoding CRF receptors in brain and pituitary of rat and mouse. *J Comp Neurol* 2000;**428**:191–212

85 Wolak ML, DeJoseph MR, Cator AD, Mokashi AS, Brownfield MS, Urban JH. Comparative distribution of neuropeptide Y Y1 and Y5 receptors in the rat brain by using immunohistochemistry. *J Comp Neurol* 2003;**464**:285–311

86 Sajdyk TJ, Schober DA, Gehlert DR, Shekhar A. Role of corticotropin-releasing factor and urocortin within the basolateral amygdala of rats in anxiety and panic responses. *Behav Brain Res* 1999;**100**:207–15

87 Sajdyk TJ, Gehlert DR. Astressin, a corticotropin releasing factor antagonist, reverses the anxiogenic effects of urocortin when administered into the basolateral amygdala. *Brain Res* 2000;**877**: 226–34

88 Sajdyk TJ, Fitz SD, Shekhar A. The role of neuropeptide Y in the amygdala on corticotropin-releasing factor receptor-mediated behavioral stress responses in the rat. *Stress* 2006;**9**:21–8

89 Sheriff S, Dautzenberg FM, Mulchahey JJ, Pisarska M, Hauger RL, Chance WT, Balasubramaniam A, Kasckow JW. Interaction of neuropeptide Y and corticotropin-releasing factor signaling pathways in AR-5 amygdalar cells. *Peptides* 2001;**22**:2083–9

90 Morris MJ, Pavia JM. Stimulation of neuropeptide Y overflow in the rat paraventricular nucleus by corticotropin-releasing factor. *J Neurochem* 1998;**71**:1519–24