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5-HT_{1A} receptor knockout mice and mice overexpressing corticotropin-releasing hormone in models of anxiety

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Abstract

Pharmacological experiments have implicated a role for serotonin (5-$HT)_{1A}$ receptors in the modulation of anxiety. More recent is the interest in corticotropin-releasing hormone (CRH) system as a potential target for the treatment of anxiety disorders. However, selective pharmacological tools for the CRH system are limited, hampering research in this field. Gene targeting is a relatively new approach to study mechanisms underlying anxiety disorders. 5-HT_{1A} receptor knockout (1AKO) mice have been created on three different background strains, and two different lines of mice, overexpressing CRH (CRH-OE), have been generated. In the present review, behavioural and physiological findings reported for 1AKO mice and CRH-OE mice will be reviewed. As behavioural phenotyping is often limited to one or two approach avoidance paradigms, we extended these observations and also tested 1AKO and CRH-OE mice in a conditioned fear paradigm. This paradigm reflects essentially different aspect of anxiety than approach avoidance paradigms. 1AKO mice on a 129/Sv background strain showed similar freezing as wild-type (WT) mice. In CRH-OE mice, less freezing was observed than in the corresponding wild-type mice. The fact that the anxious phenotype of these genetically altered mice seems less clear than initially reported will be discussed. Rather than studying the direct consequences of alterations in the targeted gene, 1AKO and CRH-OE mice seem very valuable to study compensatory processes that seem to have taken place in reaction to life-long changes in gene expression.

Keywords: Anxiety; 5-HT_{1A} receptor; CRF (corticotropin-releasing factor); CRH (corticotropin-releasing hormone); Transgenic; Knockout

1. Introduction

The brain γ-aminobutyric acid (GABA)-ergic system is the neurotransmitter system most strongly linked to anxiety. Since their introduction in the early 1960s, benzodiazepines are still the most widely prescribed compounds in the treatment of anxiety disorder. Over the years, other neurotransmitter systems have received much attention as potential targets in the search for new anxiolytics with fewer side effects. The serotonergic (5-HT) system is one of the neurotransmitter systems associated with anxiety (Griebel, 1995). Research has focussed especially on the 5-HT_{1A} receptor subtype, but so far, this has resulted in only few clinically effective compounds. More recent is the interest in the neuropeptide corticotropin-releasing hormone (CRH). This neuropeptide is known to be a key modulator of behavioural, autonomic, and neuroendocrine responses to stress (Dunn and Berridge, 1990). Persistent changes in central nervous system CRH may underlie the development of mood and anxiety disorders (Heim and Nemeroff, 1999). However, lack of highly selective agonists and antagonists for both CRH receptor subtypes has hampered research into the role of CRH in anxiety. In the last decade, genetically altered mice have been generated to further study the role of neurotransmitter systems in fear and anxiety (for reviews, see Belzung, 2001; Holmes, 2001). By selective inactivation of specific genes, knockout mice can be created, whereas adding extra genes to the genome results in the overexpression of a specific gene product. In this way, the function of a specific gene can be investigated. In the present paper, behavioural and physiological findings obtained with 5-

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HT_{1A} knockout (1AKO) mice and mice overexpressing CRH (CRH-OE) will be reviewed, and new data from our research group will be presented.

2. Serotonin and 5-HT_{1A} receptors

The serotonergic neurotransmitter system has one endogenous ligand, 5-HT, but is rather complex because of the existence of at least 14 5-HT receptor subtypes (Hoyer et al., 1994). The 5-HT_{1A} receptor has been well characterized pharmacologically, thanks to the availability of selective and potent agonists and antagonists. Both agonists and antagonists modulate anxiety behaviour in animals and, importantly, 5-HT_{1A} receptor agonists also modulate anxiety in humans. The partial 5-HT_{1A} receptor agonist buspirone is effective in the treatment of generalized anxiety disorder (Davidson et al., 1999), whereas the 5-HT_{1A} receptor agonist flesinoxan has been reported to worsen symptoms in patients suffering from panic disorder (van Vliet et al., 1996). The distribution of 5-HT_{1A} receptors in the brain correlates well with a function in emotional processes and is comparable between species, including man. 5-HT_{1A} receptors are present in high densities in limbic areas such as hippocampus, the septum, some of the amygdaloid nuclei, and entorhinal cortex (Pazos and Palacios, 1985). Activation of these postsynaptic 5-HT_{1A} receptors results in the inhibition of neurons of various neurotransmitter systems, includ-

Table 1
Behaviour of 1AKO mice on different background strains relative to WT mice in nonstress situations and in several anxiety paradigms

Background strain	C57Bl6/J	Mixed Swiss Webster × 129/Sv	129/Sv[a]	129/Sv[b]
Spontaneous behaviour				
Home cage activity	Normal[c]	X	Normal[d]	Normal[e]
Rotarod	Normal[c]	Normal[f]	X	X
Acoustic startle response	X	X	Normal[g]	Normal[h]
Approach avoidance				
Open field				
Time and entries in centre	Reduced[c]	Reduced[f]	Reduced[d]	Normal[i]
Total activity	Normal[c]	Normal[f]	Reduced[d]	Normal[i]
Novel object				
Time and entries in object area	Reduced[c]	X	X	X
Novelty suppressed feeding				
Latency to start eating	X	X	Increased[j]	X
Elevated (zero) maze				
Time and entries in open area	Reduced[c]	Reduced[k]	Reduced[d]	Normal[i]
Total entries	Normal[c]	X	Normal[d]	Normal[i]
Light–dark box				
Time in light	X	X	X	Normal[i]
Total activity	X	X	X	Normal[i]
Conditioned fear				
Freezing to context	X	X	X	Normal[i]
Freezing to cue	X	X	X	Normal[i]
Miscellaneous				
SIH	X	X	X	Normal[l,m]
Foot shock sensitisation	X	X	X	Normal[h]
Swim test/tail suspension				
Immobility time (day 1)	Reduced[c]	Reduced[f]	Normal[d]	Reduced[n]

X = not tested or not reported.
[a] Columbia University, New York, USA.
[b] Utrecht Institute for Pharmaceutical Sciences, Utrecht, The Netherlands.
[c] Heisler et al., 1998.
[d] Ramboz et al., 1998.
[e] Pattij et al., 2002a.
[f] Parks et al., 1998.
[g] Dulawa et al., 2000.
[h] Dirks et al., 2001b.
[i] Groenink et al., this article.
[j] Gross et al., 2000.
[k] Sibille et al., 2000.
[l] Pattij et al., 2001.
[m] Pattij et al., 2002b.
[n] Pattij, 2002.

ing GABA-ergic interneurons (Barnes and Sharp, 1999). High densities of 5-HT_{1A} receptors are also found in the raphe nuclei where all of the rostrally projecting 5-HT cell bodies are located. Activation of these somatodendritic autoreceptors reduces the firing rate of 5-HT neurons, which results in the suppression of 5-HT synthesis, turnover, and 5-HT release in the projection areas (Boess and Martin, 1994). Together with the serotonin transporter and the 5-HT_{1B} receptor, which are both located presynaptically in the synaptic cleft, 5-HT_{1A} receptors thus regulate the fine tuning of the activity of 5-HT neurons. Disturbances in this regulation may underlie various psychiatric disorders, including anxiety disorders and depression (Pineyro and Blier, 1999).

2.1. 5-HT_{1A} receptor knockout mice

5-HT_{1A} receptor knockout (1AKO) mice on different background strains were created by three independent research groups (Heisler et al., 1998; Parks et al., 1998; Ramboz et al., 1998) to study the involvement of 5-HT_{1A} receptors in anxiety and depression. Mice were tested in approach avoidance paradigms, which are based on a conflict between the innate tendency to explore a novel environment and to avoid an aversive novel place. The general finding was that 1AKO mice did not show changes in overall activity, but were selectively less active than wild-type (WT) mice in stress-like situations, which may be indicative of an anxious phenotype (Table 1; Olivier et al., 2001; Toth, this issue).

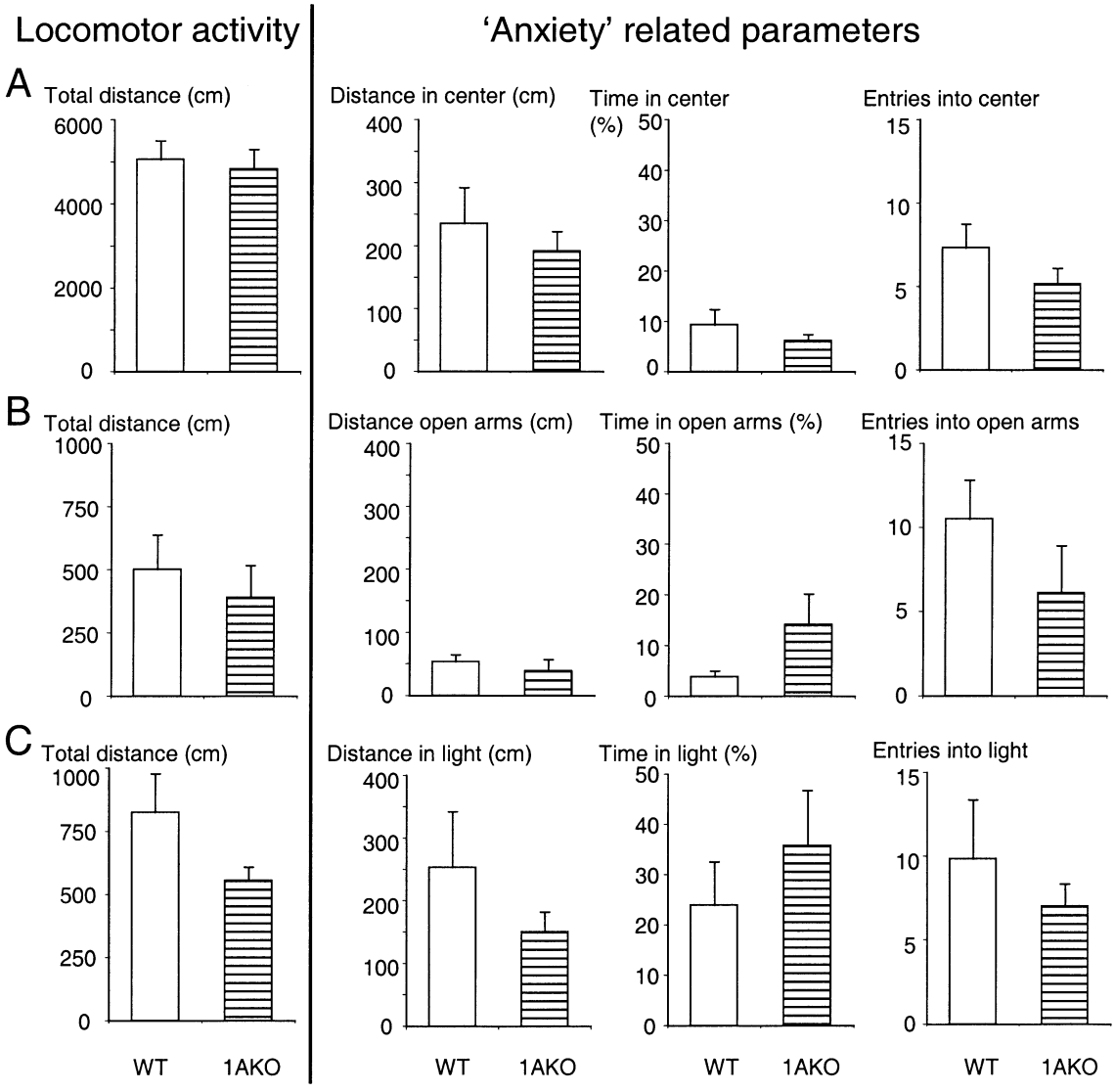

Fig. 1. Behaviour of WT and 129/Sv 1AKO mice in (A) open field, (B) elevated plus maze, and (C) light–dark box test. Data are expressed as mean + S.E.M. Mice were group-housed, 8–12 weeks old. Tests were performed during the inactive phase of the day. (A) Open field. Test duration—15 min. Diameter—75 cm, walls—35 cm high, illumination—ca. 150 lx; $n = 16$ per genotype. (B) Elevated plus maze. Test duration—5 min. Arms—30 × 5 cm, closed arm walls were 15 cm high, illumination—ca. 5 lx, $n = 8$ per genotype. (C) Light–dark box. Test duration—10 min. White compartment—ca. 850 lx, 28 × 28 × 30 cm; black compartment—ca. 50 lx, 14 × 28 × 30 cm; $n = 12$ per genotype.

Intuitively, approach avoidance paradigms, like the elevated plus-maze, have plausible face validity to investigate anxiety. However, they also have some major limitations. While benzodiazepines generally produce reliable and reproducible effects in these paradigms, the findings with 5-HT_{1A} receptor agonists are less consistent. For instance, evidence for both anxiolytic and anxiogenic effects of 5-HT_{1A} ligands in the elevated plus-maze is extensive (Belzung and Griebel, 2001; Griebel, 1995; Hogg, 1996). This indicates that these models are prone to false positive and false negatives, particularly when a drug alters locomotor behaviour (Dawson and Tricklebank, 1995). In addition, approach avoidance paradigms have been shown to be extremely sensitive to mild environmental factors (Crabbe et al., 1999). Regarding these limitations, it becomes clear that studying knockouts and transgenics in these paradigms requires caution when results are to be interpreted. It is commonly thought that 5-HT_{1A} receptor agonists are less effective in anxiety models measuring conditioned responses than in models measuring unconditioned responses, like approach avoidance paradigms, but this is not the case (Griebel, 1995). Studying behaviour of 1AKO mice in both approach avoidance paradigms and fear conditioning models could provide important information about the role of 5-HT_{1A} receptors in fear and anxiety, as the brain circuitry and systems involved in fear conditioning are well known (Davis, 1998; Fendt and Fanselow, 1999; LeDoux, 1998).

2.2. Further behavioural characterisation of 1AKO mice on a 129/Sv background

To study the effects of knocking out 5-HT_{1A} receptors on anxiety behaviour more closely, we performed the experiments described below. Besides replicating approach avoidance studies in an open field and elevated plus-maze test, we also tested 129/Sv 1AKO mice in paradigms, in which they had not been tested before, which is the light–dark box and a classical fear-conditioning paradigm.

1AKO mice and WT mice on a pure 129/Sv background were originally derived from the laboratory of Rene Hen (Ramboz et al., 1998) and bred in our laboratory. Male 1AKO mice were backcrossed on a pure 129/Sv background (M&B, Bomholt, Denmark). Heterozygous mutant mice on a pure 129/Sv background, obtained in the first generation (F1), were crossbred to obtain homozygous 1AKO and WT mice. Behaviour of WT and 129/Sv 1AKO mice was studied in an open field, in a light–dark box, and an elevated plus-maze. General locomotor activity of WT and 129/Sv 1AKO mice was similar in the three paradigms, as has been reported before (Table 1). However, in none of the three paradigms, differences were found between WT and 129/Sv 1AKO mice with regard to 'anxiety' related parameters (Fig. 1A–C). The level of exploratory activity of WT mice in the approach avoidance tests is quite low. In fact, in several approach avoidance paradigms, the 129/Sv strain has been shown to be one of the least explorative and most anxious inbred strains (Paulus et al., 1999). Decreasing low levels of activity even further by means of gene mutation is difficult and may contribute to the negative results of the present study. On the other hand, Ramboz et al. (1998) did report anxious behaviour of 129/Sv 1AKO mice despite the low activity levels of the 129/Sv WT mice. The effect of diazepam was also tested in the elevated plus-maze. Diazepam increased the percentage time and entries into the open arms, showing pharmacological sensitivity of the model (Pattij et al., 2002b). The effects of diazepam were similar in WT and 129/Sv 1AKO mice, confirming that alterations in the $GABA_A$ complex as observed in 1AKO on a mixed Swiss Webster × 129/Sv background do not occur in 1AKO mice on a 129/Sv background (Pattij et al., 2002b; Sibille et al., 2000; Toth, this isssue).

Following classical fear conditioning, mice showed increased freezing in both the context (Fig. 2A) and the

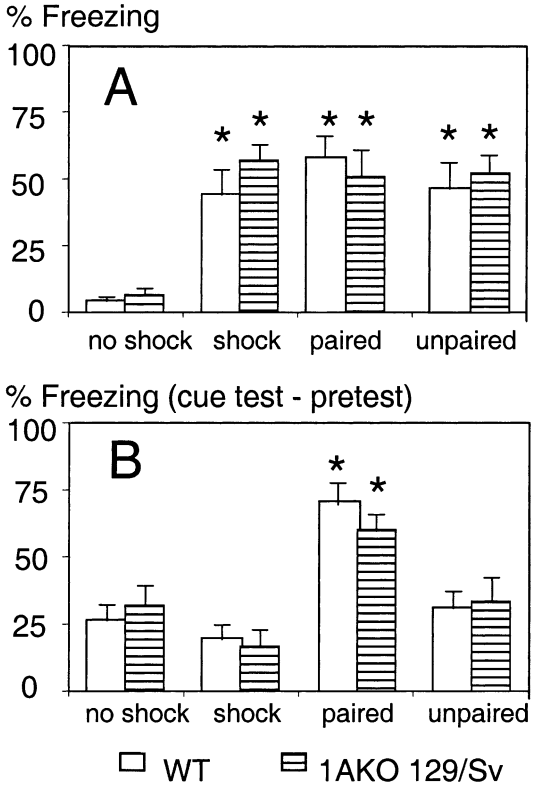

Fig. 2. Percentage time spent freezing (A) during a 5-min context test performed in the operant chamber 24 h after training, and (B) during a 6-min auditory cue test (3-min pretest no cue, 3 min cue present) performed in a modified operant chamber 25 h after training (modifications in smell, tactile, and visual stimuli). Data are expressed as mean + S.E.M. for WT (open bars, $n = 8$ per condition) and 129/Sv 1AKO mice (hatched bars, $n = 8$ per condition). *$P < 0.05$ compared to no-shock control of the corresponding genotype. The fear conditioning procedure was modified from Paylor et al. (1994) and was conducted in a mouse operant chamber. The unconditioned stimulus (US) was a 2-s, 0.7-mA foot shock. The conditioned stimulus (CS) was a 30-s pure tone (4.5 kHz, 75dB). Training conditions: no-shock, 330 s in operant chamber; shock, 2 foot shocks 150 s apart; tone paired, two CS–US pairings separated by 120 s; tone-unpaired, presentation of US and CS 40 s apart, repeated after 80 s.

auditory cue test (Fig. 2B). However, no differences were found between WT and 1AKO mice. During training, also no differences were also observed between genotypes, neither in time spent freezing (ca. 15%) nor in shock reactivity. The levels of freezing obtained on the test day are high. It could be argued that ceiling levels have been reached, thus concealing differences between genotypes. However, presentation of one foot shock during acquisition, instead of two, did result in lower freezing levels but not in differences between WT and 129/Sv 1AKO mice (data not shown). The amygdala is heavily involved in fear conditioning, both in the acquisition and expression of freezing (Fendt and Fanselow, 1999). Activation of 5-HT_{1A} receptors in the amygdala has been shown to affect conditioned fear responses (Groenink et al., 2000; Hodges et al., 1987). However, the behavioural results obtained in the present study do not suggest that the absence of 5-HT_{1A} receptors has resulted in altered modulation of behavioural output of the amygdala. The hippocampus is thought to be involved in contextual conditioning but not in conditioned freezing to discrete cues. The hippocampus is also a 5-HT_{1A} receptor-rich area. 1AKO mice have been shown to display a deficit in hippocampal-dependent learning and memory tests, such as the hidden platform, and a spatial version of the Y-maze. Performance of 1AKO mice was not impaired in non-hippocampal memory tasks (Sarnyai et al., 2000). In the fear-conditioning test, contextual conditioning of 1AKO was comparable to that of WT mice, illustrating the idea that although the hippocampus is involved in contextual learning, the amygdala is specifically involved in learning emotional aspects of fear conditioning.

There may be several environmental and experimental factors (Crabbe et al., 1999; Van der Staay and Steckler, 2001) that could account for the absence of an anxious phenotype. However, the fact that no differences were found between genotypes, neither in any of the approach avoidance paradigms nor following fear conditioning and the stress-induced hyperthermia (SIH) test which is independent of locomotor activity (Pattij et al., 2001), strongly suggests that 1AKO on a 129/Sv background do not have a clear anxious behavioural phenotype. The incongruent results are especially striking with regard to the 129/Sv 1AKO mice, from which our breeding colony was derived. The fact that the same knockout strain does not yield the same results at a different location raises questions about housing and test conditions, and also about the stability of the genetic and behavioural alterations across generations. To determine the significance of lack of 5-HT_{1A} receptors for anxiety behaviour, more extensive behavioural phenotyping of 1AKO mice on the other background strains is needed. To exclude the influence of environmental and experimental factors, we are currently breeding all three 1AKO strains in our laboratory for full comparison of 1AKO mice on different background strains.

Interestingly, both the original and the derived 129/Sv 1AKO mice show clear and reproducible exaggerated autonomic responses following mild stress (Table 2; Gross et al., 2000; Pattij et al., 2002a), whereas 24-h patterns of heart rate and body temperature in 1AKO mice do not differ from WT mice (Pattij et al., 2002a). Aversive emotional arousal and stress are often accompanied by widespread sympathetic activation both in humans and animals. In humans, a link has been proposed between low heart rate variability and risk for anxiety disorders (Friedman and Thayer, 1998a,b). Such a reduction in heart rate variability has also been observed in 1AKO mice (Pattij et al., 2002a). At the moment, it is not clear whether the autonomic hyperresponsiveness to stress observed in 129/Sv 1AKO mice is related to a heightened emotional response. It could also result from reduced inhibitory control of sympathetic activity, which is normally exerted following activation of 5-HT_{1A} receptors (McCall et al., 1989). It will be interesting to measure cardiovascular responses and behaviour simultaneously in anxiety models to further study this possible dissociation of behavioural and autonomic responses to aversive events in 1AKO mice.

Table 2
Stress-induced autonomic changes in 1AKO on a 129/Sv background, relative to WT mice

	Δ Heart rate	Δ Body temperature
Stressor		
Injection	Increased[a]	Increased[a]
Novel cage	Increased[b]	Increased[b]
Foot shock	Increased[c]	X

X = not tested.
[a] Olivier et al., 2001.
[b] Pattij et al., 2002a.
[c] Gross et al., 2000.

3. CRH and anxiety

CRH is involved in regulating adaptive responses to stress. CRH not only activates the hypothalamic pituitary adrenal axis and the autonomic nervous system, but also modulates behavioural responses to a wide range of stressful stimuli. Central CRH administration has arousing effects, including increases in heart rate and blood pressure, and increases in locomotion and acoustic startle response (Koob et al., 1993). This profile of behavioural activation shifts to enhanced suppression of behaviour in situations of stress, in which CRH increases freezing, facilitates conditioned fear, and decreases exploration and feeding (Koob et al., 1993). The distribution of CRH and its binding sites in the central nervous system can be divided into two systems. First is the neuroendocrine system, with CRH neurons originating in the parvocellular division of the paraventricular nucleus of the hypothalamus. These neurons mainly project to the external layer of the median eminence (Antoni et al., 1983). Second is the non-endocrine CRH system consisting of diffuse neuronal circuits comprising neocortical, limbic, hypothalamic, and brainstem areas (Behan et al., 1996; Van Pett et al., 2000). These brain areas are involved in modu-

lating behavioural arousal, sensory information processing, memory and learning, and autonomic regulation. For the behavioural effects of CRH, the amygdaloid nuclei and locus coeruleus seem to be particularly important. The presence of CRH immunoreactivity in the raphe nuclei and the locus coeruleus, the origins of the major serotonergic and noradrenergic pathways in the brain, respectively, points to a role of CRH in modulating these monoaminergic systems, which are known to modulate emotions.

The heterogeneous distribution of the CRH_1 and CRH_2 receptor mRNA indicates distinctive functional roles for each receptor subtype. CRH_1 receptors are highly expressed in the pituitary and specific brain areas including the cerebral cortex, septum, brainstem, and cerebellum (Behan et al., 1996; Van Pett et al., 2000). Apart from its function in the neuroendocrine system, it has been suggested that CRH_1 receptors may modulate cognitive aspects of behaviour, including attention, executive functions, anxiety, and, possibly, learning and memory (Steckler and Holsboer, 1999). In rodents, the CRH_2 receptor subtype is expressed in the form of two functional splice variants: $CRH_{2\beta}$ is mainly a peripheral receptor expressed in heart and blood vessels. $CRH_{2\alpha}$ receptors are predominantly expressed in the lateral septum, amygdala, hypothalamus, and dorsal raphe nucleus (Chalmers et al., 1995; Van Pett et al., 2000). $CRH_{2\alpha}$ receptors primarily seem to influence processes necessary for survival, including feeding, reproduction, and defense reactions (Steckler and Holsboer, 1999). The role of $CRH_{2\alpha}$ receptors in anxiety behaviour is unequivocal. Both anxiolytic and anxiogenic effects have been ascribed to this receptor based on studies with CRH_2 receptor KO mice (Bakshi and Kalin, 2000). Moreover, CRH is not the only endogenous ligand for this receptor. By now, the CRH-related peptides, urocortin, urocortin II, and urocortin III, have been identified (Lewis et al., 2001; Reyes et al., 2001; Vaughan et al., 1995). Whereas CRH is relatively selective for CRH_1 over CRH_2 receptors, urocortin binds with high affinity to both CRH_1 and CRH_2 receptors (Vaughan et al., 1995).

Considering the important role of CRH mediating the physiological and behavioural response to stress, it can be argued that disturbances in the control of CRH synthesis, CRH release, or biotransformation may compromise homeostasis as well as stress adaptation of an organism, and eventually may result in pathophysiology, including major depression and anxiety. There is a strong link between hypersecretion of CRH and major depressive disorder (Nemeroff, 1996). Clinical data also provide some evidence for an involvement of central CRH neuronal systems in anxiety disorders, but not to the extent observed in major depression (Arborelius et al., 1999; Heim and Nemeroff, 1999). CRH concentrations in the cerebrospinal fluid (CSF) are increased in certain anxiety disorders (including obsessive–compulsive disorder and posttraumatic stress disorder) but not in others (i.e. panic disorder and generalized anxiety disorder). To study the behavioural and physiological consequences of long-term central hypersecretion of CRH, transgenic mouse models of CRH overproduction were developed.

3.1. Physiological alterations in CRH-OE mice

Stenzel-Poore et al. (1992) were the first to describe the effects of CRH overproduction in transgenic mice. Those transgenics were created using a chimeric CRH transgene comprised of the murine metallothionein (mMT1) promotor driving the rat CRH gene, including introns. In the brain of mMT1 CRH-OE mice, elevated CRH expression was observed in nearly all areas normally expressing CRH. In addition, CRH overexpression was detected in several regions not previously identified as areas of normal CRH expression (Table 3). Moreover, elevated CRH mRNA was also observed in peripheral organs like heart, testis, and lungs mimicking endogenous expression of mMT1, which starts early in the embryonic development (Andrews et al., 1991). Thy1 CRH-OE mice were created using a chimeric CRH transgene comprised of the murine Thy-1.2 promotor and regulatory regions driving the rat CRH gene (Dirks et al., 2002a). This Thy1 CRH-OE overexpression model appears to be a valuable addition to the mouse model described by Stenzel-Poore et al. (1992) because overexpression starts 4 to 8 days after birth (Luthi et al., 1997) and is restricted to the central nervous system and spinal cord due to the promotor and regulatory regions used (see Table 3). There is, however, an increase in CRH-IR fibres in the adrenal medulla (Dirks et al., 2002a). Most likely, these fibres originate from preganglionic neurons in the spinal cord (Bagdy et al., 1990) and are an indirect result of central CRH overexpression (Li and McDonald, 1997). The physiological changes observed in these two independent transgenic lines are summarized in Table 3. Both lines exhibit elevated plasma corticosterone levels, although this effect is more pronounced in the mMT1 CRH-OE mice. Both CRH-OE lines show a blunted corticosterone response to stress. That is, despite elevated basal corticosterone levels, corticosterone levels after stress do not differ from those of WT mice. However, the mechanisms maintaining the hyperactivity of the hypothalamic pituitary adrenal axis seem to differ. In the mMT1 CRH-OE mice, a marked increase in plasma adrenocorticotropin hormone (ACTH) concentrations is observed, but there is no significant increase of CRH in the paraventricular nucleus of the hypothalamus. Consistent with elevated plasma ACTH and corticosterone levels, these mice developed a Cushing's syndrome-like phenotype, consisting of excess fat accumulation, muscle atrophy, thin skin, and alopecia from a very early age on (Stenzel-Poore et al., 1992). Thy1 CRH-OE mice, on the other hand, do show increased levels of CRH in the paraventricular nucleus of the hypothalamus, but only marginally elevated plasma ACTH concentrations, which could be indicative of adrenal hypersensitivity towards ACTH and downregulation of CRH_1 receptors at the level of the pituitary. Thy1 CRH-OE mice have no Cushing-like

Table 3
Physiological alterations in CRH-OE mice relative to WT mice

	CRH-OE Salk Institute for Biological Studies	CRH-OE Max Planck Institute of Psychiatry	CRH-OE Utrecht Institute for Pharmacological Sciences
Construct and promotor	Rat genomic CRH, murine metallothionein1 (mMT1) gene[a]	Rat genomic CRH, murine metallo-thionein1 (mMT1) gene[a,b]	Rat genomic CRH, murine Thy-1.2 (Thy1) gene[c]
Overexpression of CRH			
Central nervous system	In endogenous areas (but not the PVN) and non-CRH areas (e.g. granular layer dentate gyrus, and cerebellum)[a]	X	Endogenous areas (e.g. PVN, central amygdala, BNST) and non-CRH areas (e.g. granular layer dentate gyrus, and lateral habenula)[c]
Periphery	Lung, adrenal, heart, and testis[a]	X	Adrenal medulla only[c]
HPA axis			
Plasma ACTH	5-fold increase[a]	X	Marginal increase[d]
Plasma corticosterone	10-fold increase[a]	Increased[e]	5-fold increase[d]
Corticosterone after stress	Normal[f]	Normal[e]	Normal[d]
Dexamethasone suppression	X	X	Reduced[d]
Adrenal weight	Increased[a]	X	Increased[d]
Cushing-like phenotype	Early on[a]	X	Starting after 5–6 month[c]
Heart rate	X	X	Increased in light phase[c]
Body temperature	X	X	Increased end light phase[c]
Water intake	X	Increased[b]	Increased[c]
Food intake	X	X	Increased[c]
Pain sensitivity	X	Hotplate normal[b]	Hotplate normal[g]

X = not tested or not reported. PVN—paraventricular nucleus of the hypothalamus; BNST—bed nucleus of the stria terminalis.
[a] Stenzel-Poore et al., 1992.
[b] van Gaalen et al., 2002b.
[c] Dirks et al., 2002a.
[d] Groenink et al., 2002.
[e] van Gaalen et al., 2002a.
[f] Coste et al., 2001.
[g] de Jongh et al., in preparation.

phenotype. Only after 6 months increased fat deposition and hair loss become apparent. Autonomic alterations have only been studied in Thy1 CRH-OE mice. The increase in heart rate and reduction in heart rate variability fit a chronic stress-like phenotype (Dirks et al., 2002a). It is interesting to see that a basic function like water intake is increased in both CRH-OE lines. At least in Thy1 CRH-OE mice, this increase seems related to increased food intake. In general, CRH is thought to lower water and food intake (Dunn and Berridge, 1990). In the case of the Thy1 CRH-OE mice, however, increased water and food intake most probably results from an adjusted energy balance to meet increased energy demands due to increases in heart rate and body temperature (Dirks et al., 2002a). Alterations in pain sensitivity may have been expected in CRH-OE mice since central CRH administration has been suggested to induce hypoalgesia, either directly or via concomitant release of beta-endorphin (Dunn and Berridge, 1990). However, in both CRH-OE lines, pain sensitivity is normal (Table 3), suggesting that long-term elevation of CRH concentrations does not result in hypoalgesia.

In conclusion, the physiological alterations observed in CRH-OE mice are remarkably consistent between the mMT1 and Thy1 CRH-OE lines. Thus, it may be assumed that these changes are related to the life-long CRH excess induced in these mice.

3.2. CRH-OE mice and anxiety

Initially, behavioural phenotyping of the mMT1 mice was restricted to a novel open field and elevated plus-maze test. More recently, those data have been extended by a different research group and can now be compared with behavioural data obtained with the Thy1 CRH-OE mice. In all approach avoidance paradigms, a reduction in general activity is observed in CRH-OE mice relative to WT mice. This finding is very consistent between research groups (Table 4). The general reduction in locomotor activity in a novel environment in CRH-OE mice parallels the novelty-dependent hypoactivity following central CRH infusion often observed in rats. This CRH-induced reduction in locomotor activity may well reflect an adequate coping strategy, as CRH is known to enhance locomotor activity in a familiar environment (Dunn and Berridge, 1990). A novel open field test has been performed with both CRH-OE lines. Time spent in or entries made into the centre of the open field are similar for CRH-OE mice and WT mice, despite the general reduction in locomotor activity of CRH-OE mice (Table 4). Thus, the behaviour of CRH-OE mice in the open field cannot simply be interpreted as anxious behaviour. A similar pattern is observed in other approach avoidance paradigms (Table 4). In only two out of nine tests

Table 4
Behaviour of CRH-OE mice compared to WT mice

	mMT1 CRH-OE[a]	mMT1 CRH-OE[b]	Thy1 CRH-OE[c]	Remarks
Spontaneous behaviour				
Home cage activity	X	X	Normal[d]	
Novel home cage	X	X	Normal[e]	
Rotarod	X	Learning reduced[f]	X	
Acoustic startle response	X	X	Reduced[g]	
Approach avoidance				
Novel open field				
Time/entries in centre	X	Normal[f]	Normal[e]	General reduction in activity, not specific for aversive areas.
Total distance/activity	Reduced[h]	Reduced[f]	Reduced[e]	
Hole board				
Number of nose pokes	X	Normal[f]	X	
Locomotor activity	X	Reduced[f]	X	
Elevated plus maze				
Time/entries open arm	Reduced[h]	X	Normal[e]	
Total entries/activity	Reduced[h]	X	Reduced[e]	
Light–dark box				
Time in light	X	Reduced[f]	Normal[e]	start in dark compartment[f]
Total activity/transitions	Reduced[i,j]	Reduced[f]	Reduced[e]	start in light compartment[e]
Conditioned fear				
Freezing to context	X	X	Reduced[k]	
Freezing to cue	X	Reduced (1 h)[f]	Reduced (24 h)[k]	
Lick suppression	X	Normal[f]	X	
Miscellaneous				
SIH	X	Normal[l]	Normal[e]	Non-locomotor test
Behaviour after prestress				Prestress:
Open field locomotion	Reduced[h]	X	X	Social defeat
Home cage activity	X	Reduced[f]	X	1 min restraint
Foot shock sensitisation	X	X	Increased[m]	10 foot shocks
Forced Swim test				
Immobility	Normal[i]	Reduced[f]	Normal[e]	

X = not tested or not reported.
[a] Salk Institute for Biological Studies, La Jolla, CA, USA.
[b] Max Planck Institute of Psychiatry, Munich, Germany.
[c] Utrecht Institute for Pharmaceutical Sciences, Utrecht, The Netherlands.
[d] Dirks et al., 2002a.
[e] Dirks et al., 2001b.
[f] van Gaalen et al., 2002b.
[g] Dirks et al., 2002b.
[h] Stenzel-Poore et al., 1994.
[i] Heinrichs et al., 1996.
[j] Heinrichs et al., 1997.
[k] Groenink et al., this article.
[l] van Gaalen et al., 2002a.
[m] Dirks, 2001.

using approach avoidance paradigms, a specific reduction in locomotor activity and/or time spent in aversive areas is observed in CRH-OE mice (Stenzel-Poore et al., 1994; van Gaalen et al., 2002b). In both cases, this is observed in mMT1 CRH-OE mice. One of these studies is a light–dark box test (van Gaalen et al., 2002b). In this particular test, mice are placed in the dark compartment at the start of the experiment (van Gaalen et al., 2002b). Considering the reduced locomotor activity in mMT1 CRH-OE mice, this may account for the increased latency to enter the light compartment. The result of this particular light–dark test is the only indication that mMT1 CRH-OE mice originally derived from Salk Institute for Biological Studies show an anxious phenotype (Table 4). In two other tests (light–dark box), the number of transitions between the light and dark compartment is reduced in CRH-OE mice, and this is interpreted as anxious behaviour (Heinrichs et al., 1996, 1997). However, considering observations in the open field and other approach avoidance paradigms, the reduction in transitions may well reflect the novelty-induced general reduction in locomotor activity observed in CRH-OE mice, rather than specific avoidance of the aversive area. Unfortu-

nately, the light–dark box test is also the only anxiety test used by both research groups working with mMT1 CRH-OE mice. This makes it hard to conclude how consistent the findings are between laboratories and also across generations. Results of the forced swim test, for example, differ between the two laboratories (Table 4). Taken together, the findings regarding anxiety-like behaviour of both mMT1 and Thy1 CRH-OE mice in approach avoidance paradigms do not indicate that long-term hypersecretion of CRH simply results in a clear anxious phenotype.

As indicated earlier, classical fear conditioning is a well-controlled method to measure aspects of anxiety, and has the advantage that the underlying neuronal circuitry is well known. To further determine the effect of long-term CRH excess in the brain on anxiety behaviour, we studied fear conditioning in CRH-OE mice (de Jongh et al., in preparation; for procedure, see legend to Fig. 2). Thy1 CRH-OE mice showed significantly less freezing than WT mice, in both the context (Fig. 3A) and auditory cue test (Fig. 3B). Interestingly, a similar finding was reported for mMT1 CRH-OE mice, although in these mice, only conditioning to the cue was tested, and reduced freezing was only observed 1 h after training (van Gaalen et al., 2002b). The observed behaviour of CRH-OE mice is in contrast with reports on CRH-induced facilitation of freezing in mice following fear conditioning. Central CRH administration before or directly after training resulted in increased freezing during testing (Radulovic et al., 1999). Differences in pain sensitivity (van Gaalen et al., 2002b; de Jongh et al., in preparation) or higher anxiety levels during training (de Jongh et al., in preparation) do not seem to underlie the low levels of freezing in CRH-OE mice during testing. Heinrichs et al. (1996) reported that mMT1 CRH-OE mice show impaired place navigation performance in a water maze. Thus, a learning impairment might account for the discrepant result. However, both the van Gaalen et al. (2002b) study and our own data do not suggest that CRH-OE mice fail to associate the conditioned stimulus with shock. Alternatively, chronic elevation of CRH levels in the brain may result in other effects than acute CRH administration. There are only a few reports on behavioural and physiological alterations following chronic central administration of CRH. All these studies are performed in rats. Most times, rats become tolerant to the effects of centrally administered CRH. The initial effects, including reduced food intake, hyperthermia, increased motor activity, and schedule-controlled behaviour, normalise after 2 to 5 days of CRH administration (Ahlers and Salander, 1993; Buwalda et al., 1997; Krahn, 1990; Linthorst et al., 1997). Centrally induced effects of CRH on the hypothalamic pituitary adrenal axis and on immune function may be longer lasting, although this is not unequivocal (Bruhn et al., 1984; Cunningham et al., 1988; Hauger et al., 1993; Hotta et al., 1991; Linthorst et al., 1997). In adult mice of both CRH-OE lines, long-term behavioural changes are apparent. Apart from the changes listed in Tables 3 and 4, these changes also include reduced prepulse inhibition (Dirks et al., 2002b), impaired sexual behaviour, and learning impairment (Heinrichs et al., 1996, 1997). As such, CRH-OE mice are very interesting to study as they may help to understand the underlying processes involved in pathological conditions in which enhanced release of endogenous CRH has been implicated, like major depressive disorder. However, considering the results obtained with CRH-OE mice in anxiety models, long-term hypersecretion of CRH does not seem to result in marked anxiogenic behaviour. Similarly, CRH knockout mice show normal stress-induced behaviour (Weninger et al., 1999). These findings may suggest that CRH has a modulating rather than an essential role in stress-induced behavioural responses and may act in concert with other endogenous CRH-like ligands. Interesting in this respect is the upregulation of urocortin in CRH KO mice (Weninger et al., 2000), whereas urocortin is downregulated in Thy1 CRH-OE mice (Kozicz et al., in preparation). Urocortin has been hypothesized to be the endogenous ligand for the CRH_2 receptors (Koob and Heinrichs, 1999; Van Pett et al., 2000), but binds to CRH_1 receptors equally well. Urocortin may contribute to the organization of the

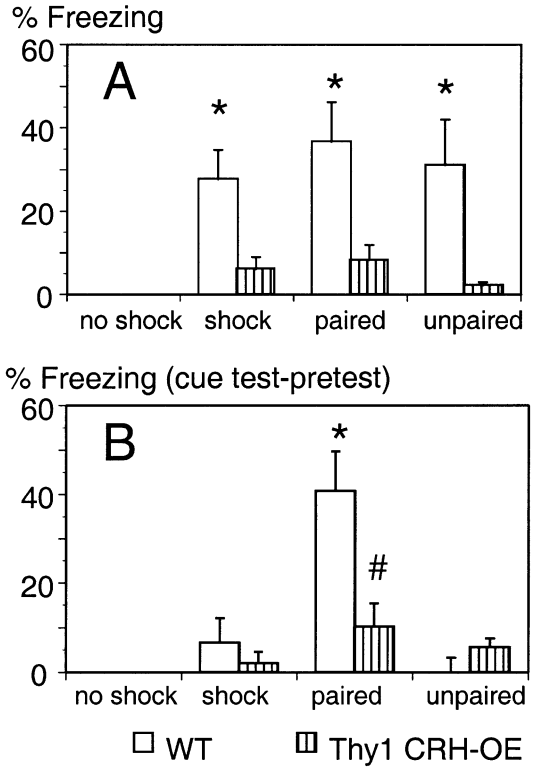

Fig. 3. Percentage time spent freezing during (A) a 5-min context test performed in the operant chamber 24 h after training and during (B) a 6-min auditory cue test performed in a modified operant chamber 25 h after training (modifications in smell, tactile, and visual stimuli). Mice were group-housed and 10–16 weeks old. Data are expressed as mean + S.E.M. for WT (open bars, $n = 6$ per condition) and Thy1 CRH-OE mice (hatched bars, $n = 6$ per condition). *$P < 0.05$ compared to no-shock control of corresponding genotype. #$P < 0.05$ compared to WT mice in same training condition. For procedure, see legend in Fig. 2.

stress response. Projections of urocortin immunoreactivity have been observed throughout the brain and spinal cord, the lateral septum, supraoptic area, PVN, central and peri-aquaductal grey, and Edinger–Westphal nucleus (Kozicz et al., 1998), although in situ hybridisation studies suggest a more limited distribution (Vaughan et al., 1995; Weninger et al., 1999, 2000; Wong et al., 1996). Coste et al. (2001) put forward a model in which CRH_1 receptors are involved in the initiation of the stress response and CRH_2 receptors play a role during the recovery phase. Interesting is that all three research groups report enhanced stress-like behaviour after prestress in CRH-OE mice compared to WT mice (Table 4). According to the proposed model, this may suggest alterations in CRH_2 receptor functioning and urocortin involvement. We are currently looking into the relative importance of CRH and urocortin in the behavioural and physiological response to stress.

As CRH-OE mice have life-long changes in gene expression, compensatory changes may also have occurred in other neurotransmitter systems. One likely candidate is the serotonergic system, which is probably one of the players in the aetiology of anxiety and depression. Chronic administration of CRH affects the serotonergic system (Linthorst et al., 1997). Moreover, elevated corticosterone levels may result in downregulation of $5\text{-}HT_{1A}$ receptors (Meijer and de Kloet, 1998). Thus, there are at least two pathways by which the serotonergic system may adapt to (indirect effects of) elevated CRH levels. A first study seems to confirm that alterations at the level of the $5\text{-}HT_{1A}$ receptor do occur in CRH-OE mice (van Gaalen et al., 2002a). A similar process may occur in humans suffering from affective disorders (Lesch et al., 1990). Apart from studying the interaction of CRH with other neurotransmitter systems, CRH-OE mice may also be valuable to study the relative importance of hypersecretion of CRH versus elevated glucocorticoid levels in behaviour, neurotransmission, and HPA axis regulation.

4. Conclusions

The present review shows that reports on anxiety behaviour in 1AKO and CRH-OE mice are not conclusive. Some research groups observed enhanced anxiety behaviour in approach avoidance paradigms, but this could not always be replicated in other approach avoidance studies or in other laboratories. Anxiety tests modelling other aspects of anxiety, like stress-induced hyperthermia, which is independent of locomotor activity, and fear conditioning both yielded negative results in 1AKO mice as well as in CRH-OE mice. Therefore, it cannot be concluded that knocking out $5\text{-}HT_{1A}$ receptors or inducing life-long CRH hypersecretion simply results in a strong anxious phenotype. Changes observed in physiology seem more consistent. Especially reports on physiological alterations in the two CRH-OE lines are quite similar. This strengthens the idea that the observed changes are related to the genetic alteration induced. It is not clear why the physiological studies are more consistent than the behavioural reports. It cannot be excluded that this can simply be accounted for by differences in sensitivity of the methods and models used. However, it may also be possible that physiological processes are more sensitive to genetic alterations than behavioural responses. The behavioural repertoire of an animal to cope with an aversive situation may be more extensive, or the possibilities to adjust neuronal pathways underlying behavioural responses may be larger.

A general problem regarding studies with genetically modified mice is the fact that the hypothesis under study is frequently based on knowledge obtained in pharmacological studies. Therefore, genetically altered mice are essentially used to confirm existing ideas. If results obtained with transgenic mice do not match previous pharmacological findings, this does not result in the rejection of the hypothesis. In such a case, concerns are raised about compensatory changes, behavioural test conditions, or background strains, or about the fact that a single gene dysfunction cannot model a complex psychiatric disorder. The same problem arises with the 1AKO and CRH-OE mice. Several behavioural findings obtained with these mice are at variance with pharmacological studies. However, pharmacological studies are often limited to acute effects of ligands, which contrasts the long-term changes induced in genetically altered mice. Moreover, pharmacological studies with $5\text{-}HT_{1A}$ receptor agonists and antagonists are not unequivocal (Griebel, 1995; Griebel et al., 1999; Groenink et al., 1996; Joordens et al., 1998). Similarly, central administration of urocortin elicits many of the same behavioural effects as CRH (Koob and Heinrichs, 1999). Thus, CRH-like peptides may actually mediate some of the effects originally ascribed to CRH. Considering clinical data, the minor changes in anxiety behaviour of 1AKO and CRH-OE mice are not too surprising. The introduction of $5\text{-}HT_{1A}$ receptor agonists has not resulted in a revolution in the treatment of anxiety disorders. In addition, although there are some indications for involvement of central CRH neuronal systems in anxiety disorders, this is limited to a few disorders (Arborelius et al., 1999).

What should be concluded from the limited changes in anxiety behaviour in the genetically altered mice reviewed here? Is the genetic model not suitable or are we putting too much emphasis on pharmacological studies? To get the most out of these genetic models, we may actually want to study the related consequences of life-long changes in the targeted gene. Studies with 1AKO mice have resulted in renewed interest in the interaction between 5-HT and GABA. Similarly, CRH-OE mice may be valuable in studying the relationship between CRH, urocortin, and serotonin, and in investigating the relative importance of elevated CRH secretion versus increased glucocorticoid levels in alterations in behavioural and physiological responses. In conclusion, both positive and negative findings should be taken into account when developing and testing concepts about the pathophysiology of anxiety disorders. Together,

advanced gene targeting techniques and pharmacological approaches may result in a better understanding of processes underlying anxiety disorders.

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