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Noradrenaline systems in the hypothalamus, amygdala and locus coeruleus are involved in the provocation of anxiety: basic studies

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Abstract

A variety of stressful events, including emotional stress, cause a marked increase in noradrenaline release in several brain regions, and especially in the hypothalamus, amygdala and locus coeruleus, in the rat brain. These findings suggest that an increased noradrenaline release could be closely related to the provocation of negative emotions such as anxiety and/or fear. In order to confirm this hypothesis, we carried out several studies. Diazepam, a typical benzodiazepine anxiolytic, significantly attenuated not only the immobilization stress-induced increase in noradrenaline release in the three rat brain regions but also the emotional changes of these animals, and these effects were antagonized by flumazenil, a benzodiazepine antagonist. Naloxone and opioid agents, such as morphine, β-endorphin and [Met^5]-enkephalin, significantly enhanced and attenuated the stress-induced increase in noradrenaline release in these regions and the stress-induced emotional change, respectively. Two stressful events which predominantly involve emotional factors, i.e., psychological stress and conditioned fear, caused significant increases in noradrenaline release selectively in these three brain regions and these increases were also significantly attenuated by pretreatment with diazepam in a flumazenil reversible manner. Yohimbine, an α_2-adrenoceptor antagonist which caused a marked increase in noradrenaline release in the several brain regions, had an anxiolytic action in the two behavioral tests involving anxiety, i.e., the conditioned defensive burying test and the modified forced swim test. β-Carbolines, which possess anxiogenic properties, significantly increased noradrenaline release in the hypothalamus, amygdala and locus coeruleus. Taken together, these findings suggest that the increased release of noradrenaline in the hypothalamus, amygdala and locus coeruleus is, in part, involved in the provocation of anxiety and/or fear in animals exposed to stress, and that the attenuation of this increase by benzodiazepine anxiolytics acting via the benzodiazepine receptor/GABA$_A$ receptor/chloride ionophore supramolecular complex may be the basic mechanism of action of these anxiolytic drugs.

Keywords: Anxiety; Stress; Noradrenaline; Hypothalamus; Amygdala; Locus coeruleus; Benzodiazepines; Opioid agent; Anxiolytic drug

1. Introduction

An increasing number of patients suffer from the anxiety disorders. Recent advances in neurochemistry, psychopharmacology and behavioral pharmacology have revealed the neurochemical mechanisms underlying not only the provocation of anxiety and but also the anxiolytic actions of benzodiazepine anxiolytics and some 5-HT$_{1A}$ receptor agonists. Brain serotonin and noradrenaline systems are thought to be involved in the provocation of anxiety. Since the discovery of benzodiazepine receptors in the late seventies (Möhler and Okada, 1977; Squires and Braestrup, 1977), studies have investigated the mechanism of action of benzodiazepines. These drugs are believed to bind to benzodiazepine receptors and to enhance GABA$_A$ receptor function, which leads to the suppression of membrane function as a result of hyperpolarization caused by an increased influx of chloride ion. However, it still remains to be determined which brain systems are affected by these drugs. In this review, based upon our previous findings, we suggest that the activity of the brain noradrenaline system, in particular, in regions such as the hypothalamus, amygdala and locus coeruleus, is in part, involved in the provocation and attenuation of anxiety and/or fear.

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2. Strategies for neurochemical and behavioral studies of anxiety in animals

Some types of anti-conflict tests, such as Geller and Seifter type (1960) and Vogel type (1971), the elevated plus maze test (Handley and Mithani, 1984; Pellow et al., 1985) and social interaction test (File and Hyde, 1978), have been used to screen new anxiolytic drugs. Another strategy for investigating anxiety is to use a stressor which induces anxiety. Fig. 1 indicates a strategy for the neurochemical investigation of anxiety where stress and drugs are used. Various types of stressors involve both physical and psychological factors, as shown by immobilization stress (Tanaka et al., 1982a,b, 1983a,b) and electric shock stress (Iimori et al., 1982), etc. Another type of stressor involves psychological factors, as shown by psychological stress (Iimori et al., 1982) and conditioned fear stress. Thus, rats are exposed to stress situations which induce anxiety and/or fear and the neurochemical changes during stress are determined by measuring the levels of neurotransmitters and their metabolites in discrete brain regions or in perfusates obtained by in vivo microdialysis. Another approach is to use drugs which have been reported to cause anxiety in humans, such as yohimbine (Charney et al., 1983) and β-carbolines (Dorow et al., 1983), and then to measure neurochemical changes in animals treated with these anxiogenic drugs. When a certain neurochemical change is found to occur during provocation of anxiety, it should be confirmed whether this neurochemical change can be reduced or abolished by the administration of anxiolytic drugs and whether this change is associated with any behavioral changes in the animals.

3. Strategies for establishing the noradrenaline hypothesis of anxiety

While physical factors are involved in immobilization stress, negative emotions, such as anxiety and/or fear, may be provoked because immobilized animals show emotional responses such as defecation, vocalization, and struggling. Immobilization stress has been reported to cause significant and marked increases in noradrenaline release in several brain regions (Tanaka et al., 1982a,b, 1983a,b, 1985a,b; Glavin et al., 1983). This has been confirmed by intracerebral microdialysis in the hypothalamus (Yokoo et al., 1990a; Pacak et al., 1992), the basolateral nucleus of the amygdala (Tanaka et al., 1991a,b), and hippocampus (Abercrombie et al., 1988). Among several brain regions studied, the increase in noradrenaline release was shown to be very marked in the hypothalamus, amygdala (Tanaka et al., 1982a,b, 1983a,b), and locus coeruleus regions (Tanaka et al., 1985a,b). The fact that stress-induced increases in noradrenaline release in the rat brain are greatly affected by various psychological factors (Tanaka, 1999; Tanaka et al., 1999), such as stressor controllability (Tsuda and Tanaka, 1985;Tsuda et al., 1986a), stressor predictability (Tsuda et al., 1989a), expression of aggression (Tsuda et al., 1988c; Tanaka et al., 1998), aging (Ida et al., 1984; Wu et al., 1997, 1999), stressor cyclicity (Shimizu et al., 1994), and activity-stress (Tsuda et al., 1982, 1983; Tsuda and Tanaka, 1990), suggests that negative emotions such as anxiety and/or fear may be caused by these stressful situations. As mentioned above, rats exposed to stress show negative emotional responses such as vocalization, defecation and struggling, which are considered to be good indices of anxiety and/or fear in animals. These findings raise the possibility that an increased release of noradrenaline in specific brain regions (hypothalamus, amygdala, locus coeruleus) is the basic neurochemical mechanism for the provocation of anxiety and/or fear in rats during exposure to stress. Fig. 2 indicates a strategy to confirm this hypothesis.

4. Main methods

In all experiments, male Wistar rats weighing around 200 g were used. The rats were exposed to various stresses

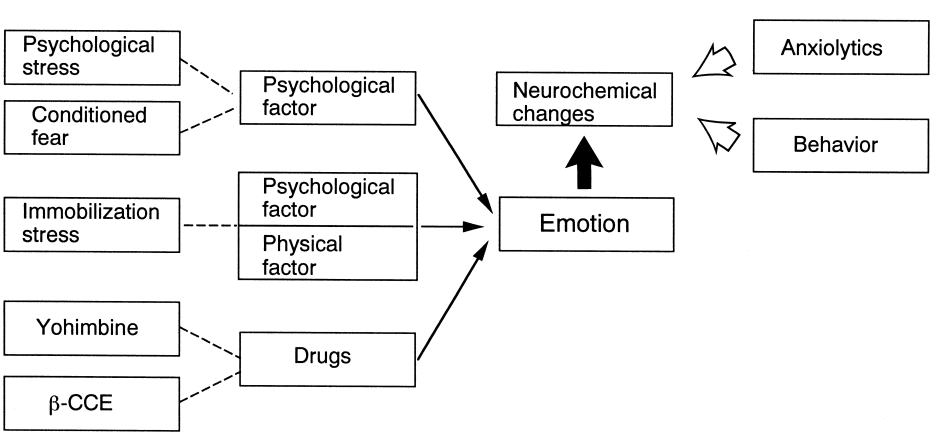


Fig. 1. Strategies for the neurochemical study of anxiety in animals.

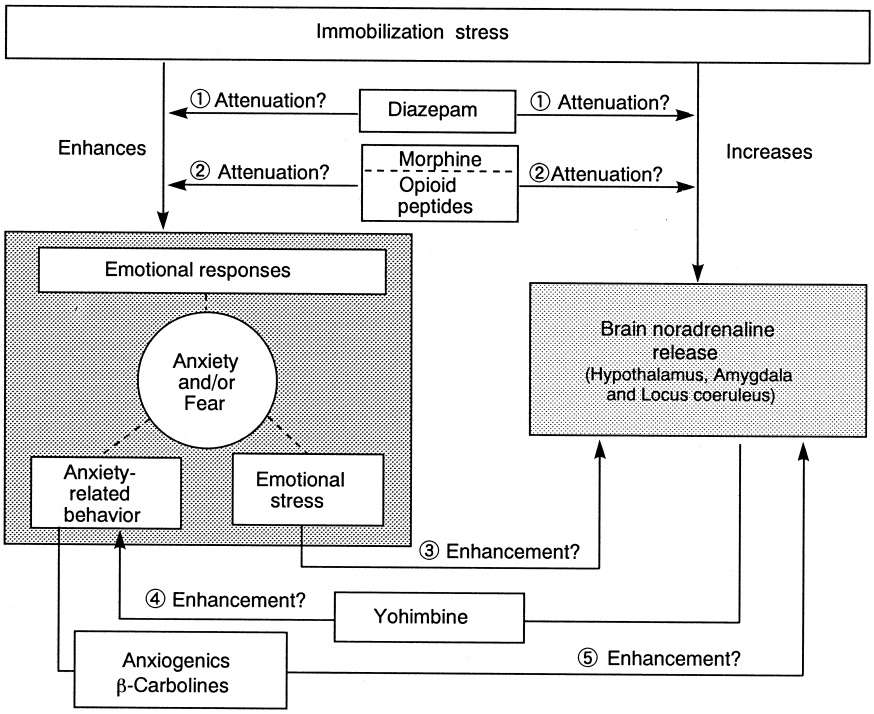


Fig. 2. Strategies to establish the noradrenaline hypothesis of anxiety.

and killed by decapitation. The brain was removed rapidly and dissected on ice into the discrete brain regions according to the method of Gispen et al. (1972), excluding the locus coeruleus region, which was dissected out by the method of Reis and Ross (1973). The levels of noradrenaline and 3-methoxy-4-hydroxyphenylethyleneglycol sulfate ($MHPG\text{-}SO_4$), the major metabolite of noradrenaline in the rat brain, in the discrete brain regions were simultaneously determined by a fluorometric method (Kohno et al., 1979).

In the intracerebral microdialysis studies, the rats were anesthetized with pentobarbital and the microdialysis probe was implanted into the anterior hypothalamus (Yokoo et al., 1990a,b) or the lateral and basolateral nuclei of the amygdala (Tanaka et al., 1991a,b, 1998). All experiments were started 24 h after surgery. Noradrenaline levels in the perfusates were determined by high-performance liquid chromatography with chemical detection.

In the behavioral investigations, defecation, vocalization, struggling, and weight loss during stress exposure were recorded as indices of negative emotional changes (Tanaka et al., 1983a; Ida et al., 1985). In order to determine the level of anxiety in the rats, two behavioral tests for anxiety were used, i.e., the conditioned defensive burying test (Tsuda et al., 1988a,b, 1989b) and the modified forced swim test (Nishimura and Tanaka, 1992; Nishimura et al., 1988a,b, 1989, 1990, 1993).

All animal procedures were performed in accordance with the Guiding Principles for the Care and Use of Laboratory Animals, approved by The Japanese Pharmacological Society and approved by the Committee of Animal Experimentation, Kurume University School of Medicine.

5. Brain noradrenaline release and anxiety

5.1. Effects of diazepam on stress-induced increases in noradrenaline release in brain regions and on emotional responses of the rats exposed to stress

In Fig. 2, the first question is whether diazepam, a typical benzodiazepine anxiolytic, can attenuate not only the increase in noradrenaline release in brain regions such as the hypothalamus, amygdala and locus coeruleus caused by immobilization stress but also the negative emotional responses of the animals to stress and, if so, whether these actions are mediated by benzodiazepine receptors. In vehicle-treated rats, immobilization stress for 1 h caused a significant increase in $MHPG\text{-}SO_4$ levels in all the nine brain regions examined (hypothalamus, amygdala, hippocampus, cerebral cortex, locus coeruleus region, thalamus, pons + medulla oblongata, midbrain, and basal ganglia) (Tanaka et al., 1982a,b, 1983a; Ida et al., 1985). Pretreatment with diazepam at 2 and 5 mg/kg i.p. significantly attenuated this increase in metabolite levels caused by stress in the hypothalamus, amygdala, hippocampus, cerebral cortex, and locus coeruleus region in a dose-dependent manner as compared to the effect of vehicle (Ida et al., 1985). Further, the attenuating effect of diazepam at

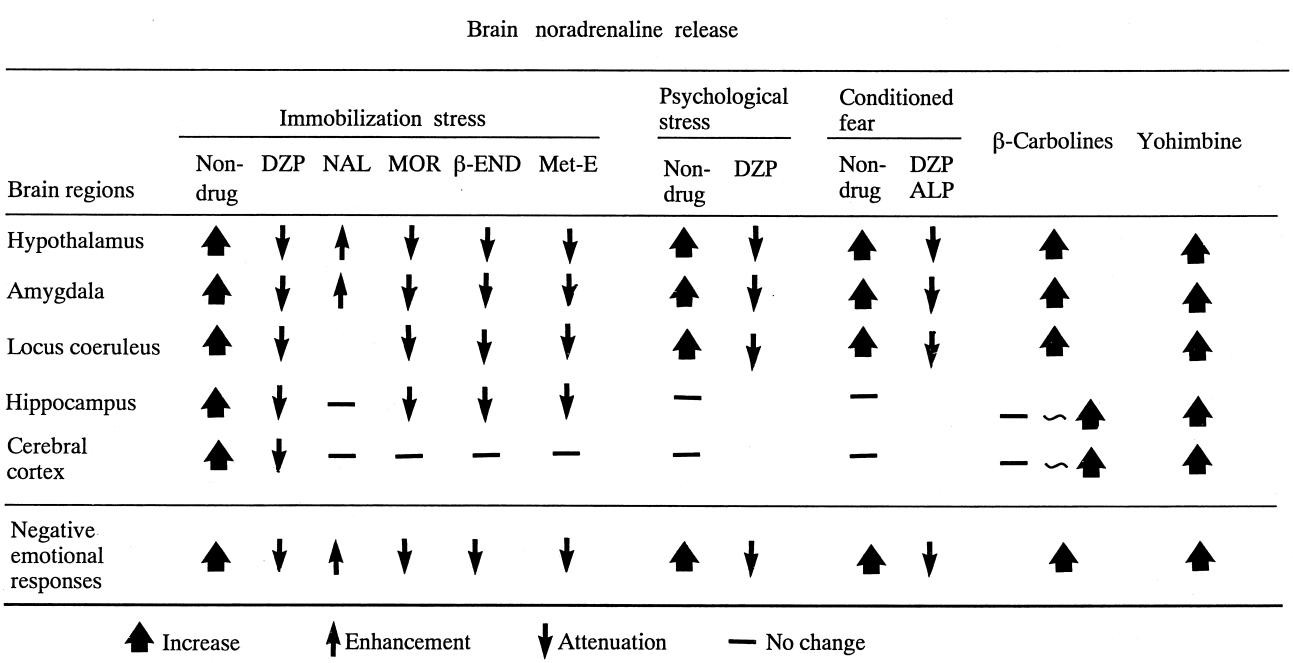
5 mg/kg in these five regions was completely antagonized by pretreatment with 5 or 10 mg/kg of flumazenil, a benzodiazepine antagonist (Ida et al., 1985). However, neither dose of flumazenil by itself affected the stress-induced increase in MHPG-SO_4 levels in all five brain regions (Ida et al., 1985). Diazepam at 5 mg/kg significantly decreased the frequency of vocalization during the first 30 min of the 1-h immobilization stress period and significantly decreased the number of feces deposited during the 1-h immobilization period (Ida et al., 1985). These effects of diazepam on emotional responses were significantly antagonized by flumazenil at 10 mg/kg, although flumazenil alone did not affect the number of vocalizations or defecations (Ida et al., 1985). These behavioral results are consistent with the finding that anxiety-like behavior caused by electrical stimulation of the locus coeruleus in the monkey is reduced by diazepam (Redmond, 1977, 1979, 1987; Redmond and Huang, 1979). In the intracerebral microdialysis study, we confirmed that the increase in noradrenaline release in the anterior hypothalamus was significantly attenuated by pretreatment with diazepam at 5 mg/kg i.p. before stress exposure in a flumazenil-reversible manner (Yokoo et al., 1991).

These findings suggest that the stress-induced increase in noradrenaline release in brain regions such as the hypothalamus, amygdala and locus coerulerus is closely related to the provocation and that the attenuating effects of diazepam on this increase might be related to the anxiolytic property of the drug (Table 1).

5.2. Effects of opioid agents on stress-induced increases in noradrenaline release in the hypothalamus, amygdala, thalamus, hippocampus, midbrain, locus coeruleus region, and emotional responses of the rats exposed to stress

We have reported that the increase in MHPG-SO_4 levels caused by immobilization stress is significantly enhanced in the hypothalamus, thalamus and amygdala by pretreatment with naloxone at 5 mg/kg, an opioid antagonist, and suggested that opioid peptides released during immobilization stress attenuate stress-induced increases in noradrenaline release in these regions (Tanaka et al., 1982b). Furthermore, morphine, a potent analgesic opiate, possesses anxiolytic properties in patients suffering from severe pain. The anxiolytic mechanism of opioid agents is considered different from that of benzodiazepine anxiolytics. In Fig. 2, the second question is whether opioid agents, including morphine and some opioid peptides, can attenuate not only stress-induced increase in noradrenaline release but also emotional responses shown during stress exposure. Pretreatment with morphine at 3 or 6 mg/kg significantly attenuated the increase in MHPG-SO_4 levels caused by 1-h immobilization stress in brain regions such as the hypothalamus, amygdala, thalamus, hippocampus,

Table 1
Changes in brain noradrenaline release and behavior caused by various stresses and anxiogenic drugs and their modifications by drugs



and midbrain, and these effects were significantly antagonized by pretreatment with naloxone at 0.5 or 5 mg/kg (Tanaka et al., 1983a). Moreover, emotional responses, such as struggling (activity counts/h), vocalization (frequency/h), defecation (number of feces/h) and weight loss, were also significantly attenuated by pretreatment with morphine at 6 mg/kg, and this attenuating effect of morphine was significantly antagonized by naloxone at 5 mg/kg (Tanaka et al., 1983a). We also reported that [Met^5]-enkephalin at 100 μg/rat or β-endorphin at 10 μg/rat injected i.c.v. before exposure to immobilization, stress significantly attenuated the stress-induced increase in MHPG-SO_4 levels in the hypothalamus, amygdala, thalamus, hippocampus, locus coeruleus region, and midbrain (Tanaka et al., 1985a,b, 1986, 1989, 1990a). These peptides also significantly attenuated the emotional changes induced by stress. Pretreatment with naloxone at 5 mg/kg significantly enhanced the stress-induced increase in noradrenaline release and the emotional responses of the stressed rats such as struggling and defecation (Tanaka et al., 1982b, 1985b, 1986, 1988, 1990a).

These findings suggest that morphine and opioid peptides, such as β-endorphin and [Met^5]-enkephalin, attenuate both the stress-induced increase in noradrenaline release in the brain and the emotional responses elicited by exposure to stress (Table 1).

5.3. Effects of mainly emotional stress on stress-induced increases in noradrenaline release in the brain

In order to investigate the effects of emotional stress (the third question), we used the two paradigms, which mainly evoke emotional stress, i.e., psychological stress (Iimori et al., 1982; Tanaka et al., 1990b,c 1991a,b; Tsuda et al., 1986b) and conditioned fear stress (Sueyoshi, 1989; Tanaka et al., 1990b,c).

Stress was induced in a communication chamber, an apparatus which was originally used for mice (Ogawa and Kuwahara, 1966), but which was modified for use with rats. The box measured $93 \times 99 \times 53$ cm and the floor composed of 0.3-cm stainless steel rods placed 1.3 cm, center to center. The chamber was subdivided into 25 smaller compartments (18×19 cm) by the use of transparent plastic walls. The 21 rats of the foot shock group were placed into the compartments individually and an electric shock was delivered for 1 h. The four rats of the psychological stress group were placed in non-shock compartments where plastic plates were placed on the grids to prevent the rats from receiving the foot shock. Thus, these rats did not receive an electric shock but were exposed to the emotional responses, such as defecation, urination, struggling, and jumping, shown by the rats received the foot shock (Iimori et al., 1982). In the conditioned fear stress paradigm, the rats were given an inescapable electric shock for 1 h in the shock box and 24 h later they were placed in the shock box but were not given an electric shock (Sueyoshi, 1989; Tanaka et al., 1990b,c).

Both stresses for 1 h caused significant increases in MHPG-SO_4 levels preferentially in the hypothalamus, amygdala and locus coeruleus region (Iimori et al., 1982; Tanaka et al., 1990b,c, 1991a,b; Sueyoshi, 1989; Tsuda and Tanaka, 1990). A microdialysis study further confirmed that conditioned fear increased noradrenaline release in the anterior hypothalamus (Yokoo et al., 1990b). Furthermore, the increase in noradrenaline release caused by these stressors was also attenuated by pretreatment with diazepam at 2 and 5 mg/kg and alprazolam at 2 and 5 mg/kg in a dose-dependent manner. The effects of diazepam were significantly antagonized by flumazenil (Sueyoshi, 1989; Tanaka et al., 1991a,b).

These results suggested that stress with a predominantly emotional component increases noradrenaline release preferentially in the hypothalamus, amygdala, and locus coeruleus, and that this increase can be significantly attenuated by diazepam or alprazolam in a flumazenil-reversible manner (Table 1).

5.4. Effects of yohimbine on anxiety-related behavior in rats

Yohimbine, an α_2-adrenoceptor antagonist, is reported to increase noradrenaline release in several brain regions including the hypothalamus, amygdala, and locus coeruleus (Oguchi, 1988; Ishii, 1994). In Fig. 2, the fourth question is whether yohimbine, which increases brain noradrenaline release, increases anxiety in the anxiety-related behavioral tests. In order to investigate the effects of yohimbine on behavior, we used two behavioral tests which elicit the anxiety. In the conditioned defensive burying test, reported by Pinel and Treit (1978) and by Treit (1985a), the rats were placed in a test chamber with bedding material on the floor and were given an electric shock with a wire-wrapped prod mounted on one end wall of the chamber. The amount of time the rats display burying responses (i.e., pushing and spraying the bedding material with snout and forepaws toward and over the shock prod) was recorded (Tsuda et al., 1988a,b, 1989b). Anxiolytic drugs such as diazepam selectively suppressed the conditioned defensive burying behavior in a dose-dependent fashion, whereas non-anxiolytic drugs such as D-amphetamine had no effect on this behavior (Treit et al., 1981). The effects of diazepam were produced through anxiolytic, rather than analgesic, actions (Treit, 1985b) and did not reflect a decrease in spontaneous activity (Blampied and Kirk, 1983). Yohimbine at 0.5 and 2 mg/kg significantly increased both the duration and the number of burying responses in a dose-dependent manner, and diazepam at 0.5 and 2 mg/kg significantly reduced these burying responses (Tsuda et al., 1988b; Tanaka et al., 1990a). An anxiogenic drug, ethyl-β-carboline-3-carboxylate (β-CCE),

at doses ranging from 0.1, 0.5, 1 and 2 mg/kg, also significantly increased defensive burying behavior as compared to the effect of vehicle (Tsuda et al., 1989b, Tanaka et al., 1990a).

Another behavioral paradigm is the modified forced-swim test with straw suspension (Nishimura et al., 1988a,b), which was originally reported as a new model sensitive for antidepressants by Porsolt et al. (1978). In this paradigm, the apparatus used was a vertical glass cylinder (height: 40 cm; diameter: 18 cm) equipped with four pieces of black rope (length: 30 cm; diameter: 0.4 cm), which were suspended from above. These ropes were made of cotton and the upper 18 cm was covered with straw (Nishimura et al., 1988b). The rats were placed in water for 5 min and immobility was measured. The rats were then provided with four pieces of straws for a further 5 min and the number of attempts at straw-climbing was measured. Straw-climbing was defined as escape-directed movements from the water and was considered to be closely related to anxiety and/or fear of the animals (Nishimura and Tanaka, 1992; Nishimura et al., 1988b, 1989, 1990, 1993). Yohimbine at 0.2 and 0.5 mg/kg and the anxiogenic drug β-CCE at 1 and 2 mg/kg significantly increased straw-climbing; however, diazepam at 0.5 and 2 mg/kg, doses which were unlikely to cause muscle relaxation and sedation, significantly decreased the number of attempts at straw climbing (Nishimura et al., 1989; Tanaka et al., 1990a).

These findings suggested that yohimbine, which increases noradrenaline release in brain regions such as the hypothalamus, amygdala and locus coeruleus, enhanced anxiety in anxiety-related behavioral tests in rats, as reported in humans (Charney et al., 1983) (Table 1).

5.5. Anxiogenic drugs and brain noradrenaline release

β-Carboline derivatives have been reported to cause anxiety in humans (Dorow et al., 1983) and anxiety-like behavioral changes in experimental animals (Skolnick et al., 1984a,b) via benzodiazepine receptors. In Fig. 2, the final question is whether anxiogenic drugs (β-carbolines) increase noradrenaline release in rat brain regions. Administration of FG 7142 (methyl-β-carboline-3-carboxamide), which has been reported to cause anxiety in humans (Dorow et al., 1983), and β-CCE significantly increased MHPG-SO_4 levels in the hypothalamus, amygdala and locus coeruleus (Ida et al., 1988; Oguchi, 1988). These findings indicate that anxiogenic drugs increase noradrenaline release in specific brain regions (Table 1).

6. The neurochemical mechanism of the provocation and reduction of anxiety

Based on these findings, we proposed a scheme to explain the neurochemical mechanism underlying the provocation of anxiety and the reduction of anxiety by some anxiolytics in Fig. 3 (Tanaka, 1992, 1993, 1997; Tanaka et al., 1990a, 1992a, 1992b, 1993). In this scheme (Fig. 3), increased noradrenaline release in the hypothalamus, amygdala and locus coeruleus is considered to be the most important event. A variety of stressful stimuli, yohimbine acting via α_2-adrenoceptors, and β-carbolines acting via benzodiazepine receptors as inverse agonists of benzodiazepines, increase noradrenaline release in the hypothalamus, amygdala, and locus coeruleus, which results in the provocation of anxiety and/or fear. Under

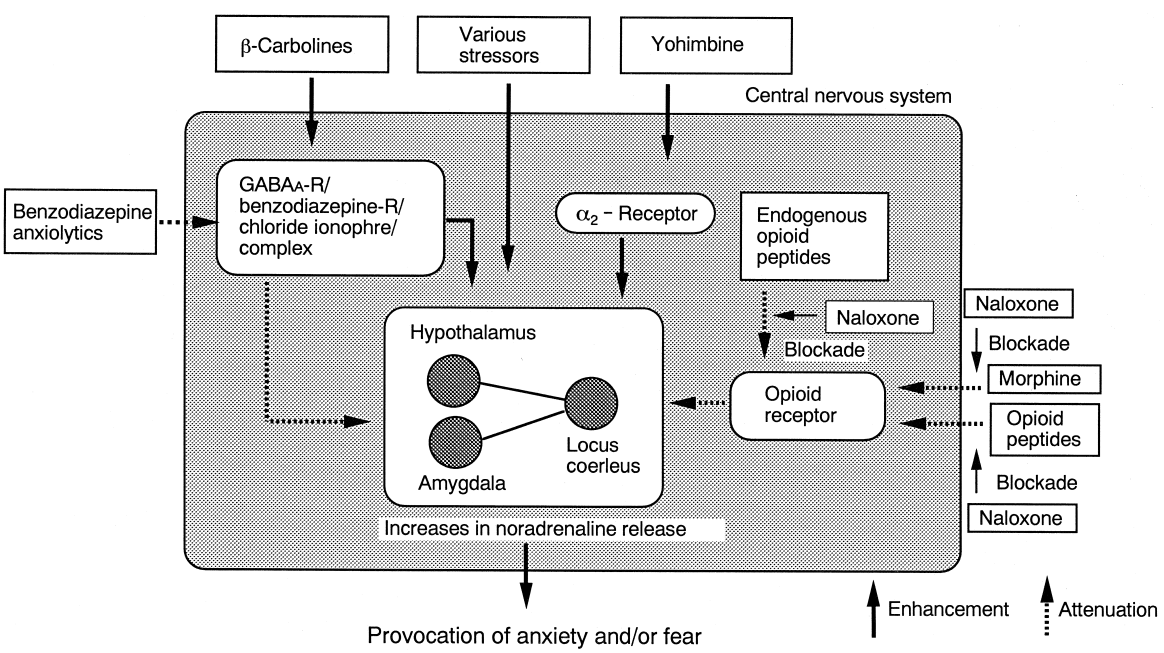


Fig. 3. Noradrenaline hypothesis proposed by the authors.

stress situations, corticotropin-releasing factor (CRF) may also have a critical role in the increased release of noradrenaline in the brain because we have found that increases in noradrenaline release in these brain regions are significantly attenuated by pretreatment with α-helical CRF, an antagonist of CRF (Emoto et al., 1993a), and that CRF injected i.c.v. significantly increases MHPG-SO_4 levels in several rat brain regions including the hypothalamus, amygdala and locus coeruleus, which suggests that CRF increases noradrenaline release in these brain regions (Emoto et al., 1993b). This was confirmed in an in vivo microdialysis study in which application of CRF through the probe significantly increased noradrenaline levels in the perfusates from the anterior hypothalamus (Emoto et al., 1993c). Arginine^8-vasopressin may also be involved, because we have found that this peptide increases noradrenaline release in the various brain regions (Tanaka et al., 1977a,b; Versteeg et al., 1978a,b).

Benzodiazepine anxiolytics acting via benzodiazepine receptors, morphine and opioid peptides such as β-endorphin and [Met^5]-enkephalin, acting via opioid receptors, μ- and δ-opioid receptors, attenuate the increases in noradrenaline release in these brain regions, leading to the relief of anxiety and/or fear. This hypothesis is basically in agreement with a report by Gray (1981) that noradrenergic input via dorsal bundles to the midbrain-hippocampal system is important for the provocation of anxiety, and with the findings that β-adrenoceptor-blocking agents are effective in the treatment of anxiety disorders (Granville-Grossman and Turner, 1966; Kathol et al., 1980), that diazepam combined with β-adrenoceptor-blocking agents is more effective than diazepam alone for the treatment of patients with chronic anxiety (Hallstrom et al., 1981), and that clonidine, an α_2-adrenoceptor agonist, is effective for anxiety disorders (Hoehn-Saric, 1982; Hoehn-Saric et al., 1981). Many hypotheses have proposed that the increase in brain noradrenaline release is involved in the provocation of anxiety (Redmond, 1977, 1979, 1987; Redmond and Huang, 1979; Charney et al., 1995; Stanford, 1995)

However, there have been reports against the noradrenaline hypothesis of anxiety (Dantzer, 1984; File et al., 1979; Mason and Fibiger, 1979; Sanghera et al., 1983; Stein et al., 1973, 1977). Moreover, a hypothesis has been advanced that the brain serotonin system has a critical role in the provocation of anxiety (File and Vellucci, 1978; Forscetti and Meek, 1981; Gallager, 1978, Graeff, 1974, 1984; Leroux and Hyers, 1975; Thiebot and Hamon, 1984; Thiebot et al., 1980). It is well known that agonists of 5-HT_{1A} receptor subtypes, such as buspirone and tandospirone, and antidepressants that selectively inhibit serotonin reuptake are clinically useful for the treatment of various anxiety disorders.

We have also reported that psychological stress increases serotonin release in the lateral and basolateral nuclei of the amygdala and prefrontal cortex (Kawahara et al., 1995) and that this increase is significantly attenuated by pretreatment with midazolam at 0.1 and 0.3 mg/kg i.v. These findings suggest that serotonin systems in the brain are also involved in the provocation of anxiety and/or fear.

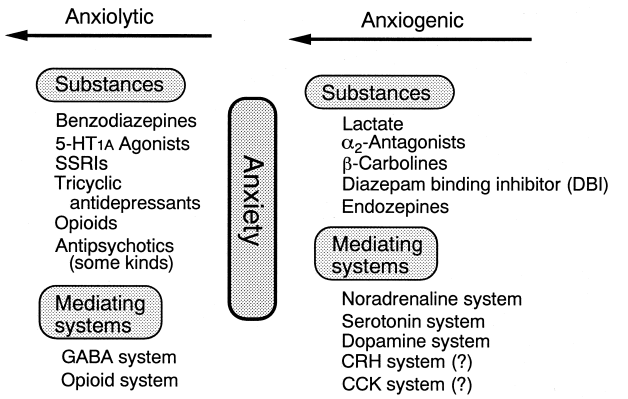


Fig. 4. Anxiogenic and anxiolytic substances and the systems mediating for the provocation and reduction of anxiety reported.

We have also found that psychological stress increases dopamine release selectively in the nucleus accumbens and the ventral tegmental area in the rat brain, and that this increase is also attenuated by pretreatment with diazepam at 5 mg/kg in a flumazenil-reversible manner (Kaneyuki et al., 1991).

It is unlikely that only one neurotransmitter or only one brain region is involved in the provocation of anxiety; rather several neurotransmitters, including noradrenaline and serotonin, and several brain regions are probably involved, as suggested by Braestrup and Nielsen (1982). Further, anxiogenic or anxiolytic substances may be involved. The systems mediating the provocation and reduction of anxiety are shown in Fig. 4. As for noradrenergic systems, an increased release of noradrenaline in the hypothalamus, amygdala and locus coeruleus is, in part, involved in the provocation of anxiety and/or fear, and the reduction of this increase may be related to the relief of anxiety in animals.

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