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Anticipation and consumption of food each increase the concentration of neuroactive steroids in rat brain and plasma

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Abstract

Stressful stimuli and anxiogenic drugs increase the plasma and brain concentrations of neuroactive steroids. Moreover, in rats trained to consume their daily meal during a fixed period, the anticipation of food is associated with changes in the function of various neurotransmitter systems. We have now evaluated the effects of anticipation and consumption of food in such trained rats on the plasma and brain concentrations of 3α-hydroxy-5α-pregnan-20-one (3α,5α-TH PROG) and 3α,21-dihydroxy-5α-pregnan-20-one (3α,5α-TH DOC), two potent endogenous positive modulators of type A receptors for γ-aminobutyric acid (GABA). The abundance of these neuroactive steroids was increased in both the cerebral cortex and plasma of the rats during both food anticipation and consumption. In contrast, the concentration of their precursor, progesterone, was increased in the brain only during food consumption, whereas it was increased in plasma only during food anticipation. Intraperitoneal administration of the selective agonist abecarnil (0.1 mg/kg) 40 min before food presentation prevented the increase in the brain levels of 3α,5α-TH PROG and 3α,5α-TH DOC during food anticipation but not that associated with consumption. The change in emotional state associated with food anticipation may thus result in an increase in the plasma and brain levels of 3α,5α-TH PROG and 3α,5α-TH DOC in a manner sensitive to the activation of $GABA_A$ receptor-mediated neurotransmission. A different mechanism, insensitive to activation of such transmission, may underlie the changes in the concentrations of these neuroactive steroids during food consumption.

Keywords: Food intake; Neuroactive steroid; Rat; Abecarnil

1. Introduction

Various stressful conditions associated with down-regulation of γ-aminobutyric acid (GABA)-mediated neurotransmission elicit anxiety-like behavior and a marked increase in the plasma and brain concentrations of progesterone and its GABA-mimetic metabolites 3α-hydroxy-5α-pregnan-20-one (3α,5α-TH PROG) and 3α,21-dihydroxy-5α-pregnan-20-one (3α,5α-TH DOC) (Barbaccia et al., 1994, 1996b, 1997; Purdy et al., 1991). Given that inhibition of GABAergic transmission induced by stressful conditions or anxiogenic drugs also results in an altered activity of various neurotransmitter systems involved in the modulation of emotional state (Dazzi et al., 1995; Finlay et al., 1995; Horger and Roth, 1996; Rueter et al., 1997), the increase in the brain content of these neuroactive steroids during stress has been interpreted as a physiological mechanism to counteract the overexcitation of neurons (Biggio and Purdy, 2001; Smith, 2003). This conclusion is consistent with the observation that 3α,5α-TH PROG and 3α,5α-TH DOC are among the most potent and efficacious positive modulators of $GABA_A$ receptors known (Belelli and Lambert, 2005; Lambert et al., 2003), eliciting marked anxiolytic, anticonvulsant, and sedative-hypnotic effects when administered in vivo (Belelli et al., 1989; Bitran et al., 1991; Finn et al., 2004; Kokate et al., 1999; Mendelson et al., 1987). Fluctuations in the brain levels of neuroactive steroids, whether associated with physiological or pathological conditions or elicited by pharmacological treatments that result in changes in emotional behavior,

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are thus a potentially useful biochemical index for evaluation of the emotional state of experimental animals. Indeed, the functional relation between stress and neuroactive steroids suggests that the regulation of both $GABA_A$ receptor function (Belelli and Lambert, 2005; Lambert et al., 2003) and expression of receptor subunit genes (Concas et al., 1998; Follesa et al., 2000, 2002; Griffiths and Lovick, 2005; Maguire et al., 2005; Smith et al., 1998) by these hormones is an important neurochemical mechanism in the modulation of emotional behavior.

The anticipation of food presentation in rats trained for several weeks to eat their daily meal within a fixed period elicits marked changes in emotional state and behavior (Holmes and Mistlberger, 2000; Inglis et al., 1994). Such behavior is associated with changes in the function of neurotransmitter systems including those mediated by GABA (Ghiani et al., 1998; Inglis et al., 1994; Merali et al., 2004). The role of this inhibitory neurotransmitter in modulation of emotions linked to ingestive behavior (Cooper and Higgs, 1996; Higgs and Cooper, 1998; Reddy and Kulkarni, 1998) together with the important inhibitory action exerted by GABA (Calogero et al., 1988) and progesterone metabolites (Toufexis et al., 2004) on the activity of the hypothalamic–pituitary–adrenal axis prompted us to investigate the effects of anticipation and consumption of food on the production of neuroactive steroids in rats. In addition, to investigate the role of $GABA_A$ receptor-mediated neurotransmission in such modulation of neuroactive steroid production, we evaluated its sensitivity to a nonsedative dose of abecarnil (Ghiani et al., 1998), a potent anxiolytic β-carboline derivative (Barbaccia et al., 1996a; Stephens et al., 1993).

2. Materials and methods

2.1. Animals and diet regimen

Male Sprague–Dawley CD rats (Charles River, Como, Italy), with body masses of 130 to 150 g at the beginning of experiments, were housed in groups of three in wire mesh-bottomed cages (7 by 12 in.). They were maintained under an artificial 12-h light, 12-h dark cycle (lights on 0800 to 2000 h) at a constant temperature of 23±2 °C and 65% humidity. After arrival at the animal facility, rats were acclimatized for a minimum of 7 days, during which time they had free access to food and water. From the second week, rats (3 per cage) were trained for 5 weeks to consume their daily food within a period of 2 h (Biggio et al., 1974); food (rat food pellets; Standard Diet GLP, Mucedola, Italy) was presented once a day at 10:00 h and removed at 12:00 h, whereas water was provided ad libitum. Rats showed a marked decrease in their rate of growth during the 1st week of training, but this parameter had returned to normal after 15 days (data not shown). At the end of each daily feeding session, the mean amount of food consumed per rat was determined from the difference between the weight of food at the start of the session and that at the end of session divided by the number of the animals. Control rats received food and water ad libitum. The day of the experiment, groups of rats ($n=8$) were sacrificed at each time point (20 min before, 20, 60, 90 min into the feeding period and 60 min after the end of the session) to measure steroid levels. Additional measurements were taken from rats fed ad libitum ($n=8$). Animal care and handling throughout the experimental procedures were in accordance with the European Communities Council Directive of 24 November 1986 (86/609/EEC).

2.2. Drug treatment

The day of the experiment, abecarnil (Schering A.G., Berlin, Germany) was dissolved in distilled water with 1 drop of Tween 80 (Aldrich, Milwaukee, WI) per 5 ml of solution and was injected intraperitoneally (0.1 mg/kg body mass/3 ml) 40 min before food presentation. Rats ($n=8$ per group) were then sacrificed 20 min before and 20 min into the feeding period. Vehicle-treated rats ($n=8$) received an equal volume of vehicle and were sacrificed at the same time points. Control rats received food ad libitum.

2.3. Extraction and assay of steroids

Rats were killed at the indicated times either with a guillotine (for measurement of plasma steroids) or by focused microwave irradiation (70 W/cm^2 for 4 s) to the head (for measurement of brain steroids). This latter procedure results in virtually instantaneous inactivation of brain enzymes, thus minimizing postmortem steroid metabolism. The brain was rapidly (<1 min) removed from the skull, and the cerebral cortices were dissected and then frozen at −20 °C until steroid extraction. Steroids were extracted and purified as previously described (Serra et al., 2000a). In brief, steroids present in cerebral cortical homogenates were extracted three times with ethyl acetate, and the combined organic phases were dried under vacuum. The resulting residue was dissolved in 5 ml of *n*-hexane and applied to a SepPak silica cartridge (Waters, Milford, MA), and components were eluted with a mixture of *n*-hexane and 1-propanol (7:3, v/v). Steroids were separated and further purified by high-performance liquid chromatography on a 5-μm Lichrosorb-diol column (250 by 4 mm; Phenomenex, Torrance, CA) with a discontinuous gradient of 2-propanol (0% to 30%) in *n*-hexane. Progesterone, which coelutes with cholesterol, was further purified by washing the corresponding dried fractions twice with 200 μl of dimethyl sulfoxide and 400 μl of water, followed by extraction from the aqueous phase twice with 1.5-ml volumes of *n*-hexane.

Blood was collected from the trunk of killed rats into heparinized tubes and centrifuged at 900×*g* for 20 min at room temperature. The resulting plasma fraction was frozen (−20 °C) until steroid extraction. Steroids were extracted from plasma three times with 1.5 ml of ethyl acetate.

The recovery (70% to 80%) of steroids through the extraction and purification procedures was monitored by adding a trace amount of tritiated standards to the brain homogenate. For the tissue extract, a mixture of [^{3}H]progesterone, [^{3}H]3α,5α-TH PROG and [^{3}H]3α,5α-TH DOC (6000 to 8000 cpm each, ~ 80 Ci/mmol) was added. Given that steroids in plasma were not subjected to chromatography, only [^{3}H]corticosterone was added to the plasma fraction. Steroids were quantified by radioimmunoassay as previously described (Serra et al., 2000a) with specific antibodies to progesterone and to corticosterone (ICN, Costa

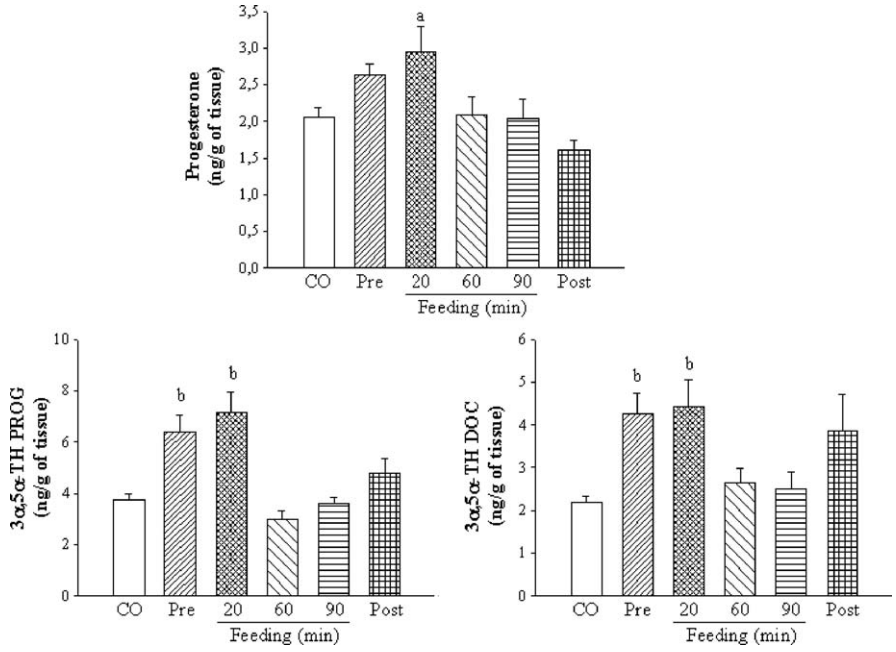

Fig. 1. Effects of anticipation and consumption of food on cerebrocortical levels of neuroactive steroids in the rat. Steroids were measured 20 min before food presentation (pre); 20, 60 and 90 min into the feeding period; 60 min after the end of the feeding section (post) and in control rats that received food ad libitum (CO). Data are expressed as nanograms of steroid per gram of tissue and are means±S.E.M. ($n=24$). [a]$p<0.05$, [b]$p<0.01$ versus control rats.

Mesa, CA) as well as with those to 3α,5α-TH PROG and to 3α,5α-TH DOC generated in rabbits and sheep, respectively, and characterized as previously described (Purdy et al., 1990).

2.4. Statistical analysis

Data are presented as means±S.E.M. and were analyzed by one-way analysis of variance. Individual means were compared by Newman–Keuls post hoc test. A $p<0.05$ value was considered statistically significant.

3. Results

Rats were trained for 35 days to consume their daily meal during a 2-h period. The amount of food consumed by each rat during the feeding period increased for the first 3 weeks of training

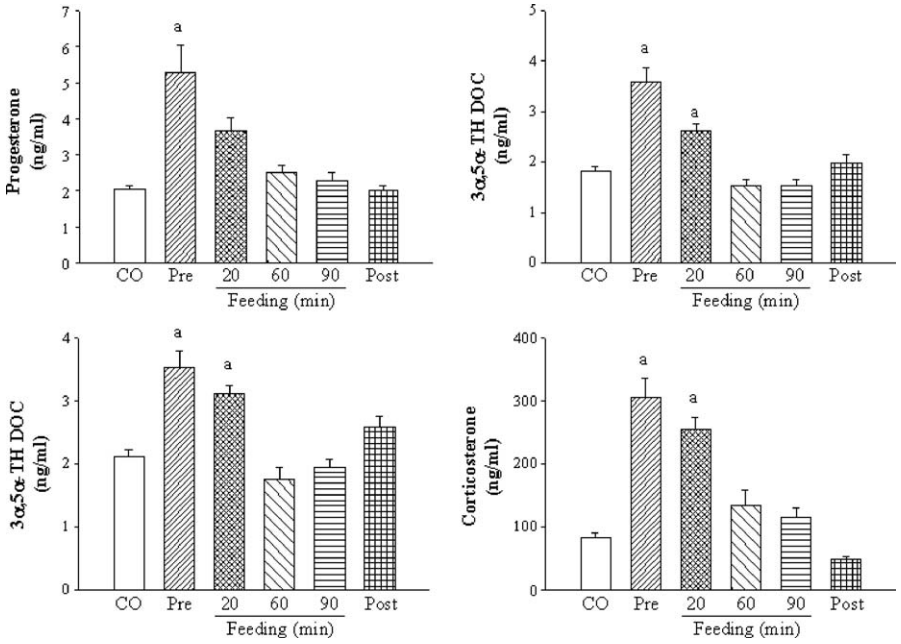

Fig. 2. Effects of anticipation and consumption of food on plasma concentrations of steroids in the rat. Steroids were measured 20 min before food presentation (pre); 20, 60 and 90 min into the feeding period; 60 min after the end of the feeding section (post) and in control rats that received food ad libitum (CO). Data are expressed as nanograms of steroid per milliliter of plasma and are means±S.E.M. ($n=24$). [a]$p<0.01$ versus control rats.

Table 1
Effects of abecarnil on the increases in the cerebrocortical levels of neuroactive steroids associated with anticipation and consumption of food

	Steroid (ng/g tissue)		
	Progesterone	3α,5α-TH PROG	3α,5α-TH DOC
Control	1.9±0.2	3.6±0.3	2.0±0.2
Vehicle (pre)	2.6±0.2	6.2±0.5[b]	4.5±0.3[b]
Abecarnil (pre)	2.0±0.2	3.5±0.4	1.8±0.3
Vehicle (during)	2.9±0.3[a]	7.0±0.6[b]	4.8±0.3[b]
Abecarnil (during)	3.0±0.2[a]	6.7±0.4[b]	5.0±0.3[b]

Abecarnil (0.1 mg/kg) or vehicle was administered intraperitoneally 40 min before food presentation. Steroids were measured 20 min before (pre) and 20 min into (during) the feeding period. They were also determined in control rats that received food ad libitum. Data are expressed as nanograms of steroid per gram of tissue and are means±S.E.M. ($n=24$). [a]$p<0.05$, [b]$p<0.01$ versus control rats.

and then remained relatively constant for the least 2 weeks (food intake: 12.1±1.0, 17.9±1.0, 19.5±0.9, 19.1±1.3 and 19.8±0.6 g/day for weeks 1 to 5, respectively; means±S.E.M., $n=24$). At the end of the training period, rats killed 20 min before the next feeding period showed increases in the cerebrocortical concentrations of 3α,5α-TH PROG and 3α,5α-TH DOC of 70% and 96%, respectively (Fig. 1). These increases were maximal during the first 20 min of food consumption (+91% and +104%, respectively) and were no longer evident 60 min into the feeding period. In contrast to its metabolites 3α,5α-THPROG and 3α,5α-TH DOC, the cerebrocortical concentration of progesterone was not significantly increased 20 min before the feeding period, but it was so 20 min after food presentation (+43%).

The plasma concentrations of 3α,5α-TH PROG and 3α,5α-TH DOC were also significantly increased (+98% and +67%, respectively) 20 min before food presentation and remained so 20 min into the feeding period, returning to basal values 1 h after food presentation (Fig. 2). In contrast to its lack of effect on the brain level of progesterone, food anticipation induced a significant increase (+160%) in the plasma concentration of this hormone, which had returned to control levels 20 min after food presentation. The plasma concentration of corticosterone was also increased (+268%) 20 min before the feeding period and remained so 20 min after food presentation, returning to baseline 1 h into the feeding period.

Consistent with previous observations (Barbaccia et al., 1996a), intraperitoneal injection of abecarnil (0.1 mg/kg) had no effect on the basal concentrations of progesterone, 3α,5α-TH PROG and 3α,5α-TH DOC in control rats (data not shown). Administration of this drug 40 min before food presentation in trained rats prevented the increase in the brain levels of 3α,5α-TH PROG and 3α,5α-TH DOC associated with food anticipation, but it did not inhibit the increase in the brain content of these neuroactive steroids and of progesterone apparent 20 min after food presentation (Table 1). Same results were obtained in plasma (data not shown).

4. Discussion

3α,5α-TH PROG is the most potent and efficacious endogenous positive modulator of $GABA_A$ receptor-mediated neurotransmission known (Belelli and Lambert, 2005; Lambert et al., 2003; Majewska, 1992). Its synthesis is increased during stress, the menstrual cycle and pregnancy as well as after the administration of antidepressant or antipsychotic drugs (Pisu and Serra, 2004). Fluctuations in the plasma and brain concentrations of this hormone thus likely influence certain pharmacological effects of such drugs and may modulate the changes in emotional state associated with these physiological conditions. Abnormalities in the synthesis of neuroactive steroids have been detected in various pathological conditions associated with altered GABAergic neurotransmission, such as ethanol withdrawal (Finn et al., 2004), premenstrual dysphoric disorder (Monteleone et al., 2000; Rapkin et al., 1997; Rasgon et al., 2001) and catamenial epilepsy (Reddy, 2004). Given that $GABA_A$ receptor-mediated inhibitory neurotransmission plays an important role in modulation of emotion, the changes in the brain levels of these endogenous regulators of $GABA_A$ receptors associated with various physiological, pathological and pharmacological conditions may affect emotional responses in affected individuals.

Pharmacological treatments that induce conflict behavior in rats and experimental anxiety in monkeys and humans (Biggio et al., 1990) also increase the peripheral and central secretion of 3α,5α-TH PROG (Biggio and Purdy, 2001). This hormone might thus function as an endogenous modulator of the threshold excitability of neurons that regulate emotional behavior, affectivity, learning and memory. Indeed, the increase in the brain content of 3α,5α-TH PROG associated with physiological anxiety has been interpreted as a compensatory mechanism to prevent excessive neuronal activation and that elicited by some antidepressants (Serra et al., 2001; Uzunov et al., 1996; Uzunova et al., 1998), atypical antipsychotics (Barbaccia et al., 2001; Marx et al., 2003) or mood stabilizers (Serra et al., 2000a) may be relevant to the therapeutic effects of these drugs.

We have now shown that, in rats trained to eat their daily meal within a fixed period of 2 h, the anticipation of food presentation is associated with a marked increase in the abundance of 3α,5α-TH PROG and 3α,5α-TH DOC in both the cerebral cortex and plasma. Moreover, the concentrations of these two hormones remain increased 20 min into the feeding period. In contrast to its metabolites, the cerebrocortical concentration of progesterone did not increase in association with food anticipation, but it did so during the first 20 min of the feeding period. The concentration of progesterone in plasma was increased only during food anticipation. This latter finding suggests that the increase in the plasma progesterone concentration associated with anticipation of food might be the consequence of HPA axis stimulation by a stressful condition. Moreover, the increased amounts of 3α,5α-TH PROG and 3α,5α-TH DOC in plasma might be the source of the increased amounts of these compounds in the brain. However, we are not able to exclude the possibility that steroidogenesis in glial cells and neurons of the brain is increased by food anticipation independently of peripheral mechanisms. Indeed, we have recently shown that ethanol elicits local production of 3α,5α-TH PROG in hippocampal slices (Sanna et al., 2004). The conversion of progesterone to its neuroactive metabolites in the brain may underlie the observation that the concentration of progesterone in the cerebral cortex, unlike that in plasma, did not increase in association with food anticipation.

Our finding that abecarnil prevented the increase in the concentrations of 3α,5α-TH PROG and 3α,5α-TH DOC in the brain during anticipation of food supports the notion that the increased production of these endogenous $GABA_A$ receptor modulators represents a physiological mechanism to counteract the increased neuronal excitability associated with the change in emotional state. The observation that abecarnil failed to inhibit the increase in the concentrations of progesterone and its metabolites in the brain elicited by food intake suggests that emotional state does not play a major role in regulation of neuroactive steroid synthesis during food consumption. The regulation and functional significance of the increases in the plasma and brain concentrations of these steroids associated with food consumption may thus differ from those of the increases associated with food anticipation.

Given that abecarnil, 3α,5α-TH PROG and 3α,5α-TH DOC are potent, efficacious and selective positive modulators of $GABA_A$ receptors, our results further suggest that $GABA_A$ receptor-mediated transmission plays an important role in the compensatory anxiolytic action of neuroactive steroids in conditions associated with an elevated emotional state. Various stressful stimuli and anxiogenic drugs reduce $GABA_A$ receptor function and increase the plasma and brain concentrations of these neuroactive steroids (Biggio and Purdy, 2001). These observations indirectly suggest that $GABA_A$ receptor function might be reduced in the brain of fasted rats.

Several studies have indicated that fluctuations in the synthesis of neuroactive steroids may reflect one of the most important mechanisms for regulation not only of the function of $GABA_A$ receptors but also of the expression of genes for $GABA_A$ receptor subunits in physiological or pharmacological conditions associated with changes in emotional state (Concas et al., 1998; Follesa et al., 2000; Griffiths and Lovick, 2005; Lovick et al., 2005; Maguire et al., 2005; Serra et al., 2000b). Given that changes in neuroactive steroid secretion are elicited by both reward expectation and consummatory behavior, two conditions linked differently to emotional state, it will be of interest to evaluate changes in $GABA_A$ receptor gene expression in brain areas involved in the regulation of emotional state in both of these conditions.

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