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

Maria Giuseppina Pisu [a,*], Ivan Floris [b], Elisabetta Maciocco [a], Mariangela Serra [a,b,c], Giovanni Biggio [a,b,c]

[a] C.N.R. Institute of Neuroscience, Cagliari, Italy
[b] Department of Experimental Biology, Section of Neuroscience, University of Cagliari, Cagliari, Italy
[c] Center of Excellence for the Neurobiology of Dependence, University of Cagliari, Cagliari, Italy

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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,

* Corresponding author. Department of Experimental Biology, Section of Neuroscience, University of Cagliari, S.S. 554 Km 4,500, 09042 Monserrato (CA), Italy. Tel.: +39 070 675 4172; fax: +39 070 675 4166.
E-mail addresses: pisu@ca.cnr.it, pisu@unica.it (M.G. Pisu).

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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