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INTRANASAL ADMINISTRATION OF PROGESTERONE INCREASES DOPAMINERGIC ACTIVITY IN AMYGDALA AND NEOSTRIATUM OF MALE RATS

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Abstract—We evaluated the effects of intranasal administration of progesterone (PROG) on the activity of dopaminergic neurons in the brain of anesthetized rats by means of microdialysis. Male Wistar rats were implanted with guide cannulae in the basolateral amygdala and neostriatum. Three to 5 days later, they were anesthetized with urethane, and dialysis probes were inserted. After a stabilization period of 2 h, four 30-min samples were collected. Thereafter, the treatment (0.5, 1.0 or 2.0 mg/kg of PROG dissolved in a viscous castor oil mixture, or vehicle) was applied into the nose in a volume of 10 μl (5 μl in each nostril). In other animals, an s.c. injection of PROG (1.0, 2.0 or 4.0 mg/kg) or vehicle was given. Samples of both application ways were collected at 30-min interval for 4 h after the treatment and immediately analyzed with high performance liquid chromatography and electrochemical detection. Intranasal administration of 2 mg/kg of PROG led to an immediate (within 30 min after the treatment) significant increase in the basolateral amygdala dopamine levels. In the neostriatum, the 2 mg/kg dose led to a delayed significant increase in dopamine. S.c. administration of 4 mg/kg of PROG was followed by a delayed significant increase in dopamine, both, in the basolateral amygdala and neostriatum, but smaller in magnitude in comparison to the intranasal treatment. This is the first study to demonstrate dopamine-enhancing effects of PROG, not only in the neostriatum, but also in the basolateral amygdala. Our results indicate that the intranasal route of administration of PROG is a more efficacious way for targeting the brain than the s.c. route.

Key words: dopamine, progesterone, basolateral amygdala, dorsal striatum, intranasal, microdialysis.

Neurosteroids are synthesized in glia cells and neurons in the CNS and peripheral nervous system, from cholesterol or from steroid hormone precursors produced by steroidogenic glands (Baulieu et al., 2001; Robel and Baulieu, 1994). Neuronal progesterone (PROG) is derived from its precursor, pregnenolone, which is derived either from the circulation or from local de novo synthesis from cholesterol. Pregnenolone is converted to PROG by 3β-hydroxysteroid dehydrogenase (Schumacher et al., 2000). This enzyme is expressed throughout the rat brain, spinal cord and dorsal root ganglia mainly by neurons (Schumacher et al., 2004). PROG is converted by 5α-reductase and 3α-hydroxysteroid dehydrogenase to several metabolites, mainly 5α-dihydroprogesterone and 3α-hydroxy-5α-pregnan-20-one (allopregnanolone), which also have PROG-like effects (Baulieu et al., 2001; McEwen, 1991; Robel and Baulieu, 1994).

The physiological effects of PROG are triggered by its binding to specific intracellular receptors that can activate transcription factors, facilitating gene expression and regulation. PROG can also act on cell membrane/cytoplasmic signal transduction pathways, inducing changes in neuronal excitability, release of neuropeptides and neurotransmitters, and modulation of neurotransmitter receptors and ion channels (Mani, 2006; Schumacher et al., 2000). There is evidence for a modulatory action of PROG and its metabolites on the activity of type A GABA ($GABA_A$), the *N*-methyl-D-aspartate (NMDA), nicotinic acetylcholine (nACh) and sigma 1 (σ1) receptors (Schumacher et al., 2000, 2007b).

Besides its well-known role in regulating reproduction and sexual behavior, PROG has been implicated in several physiological and pathological processes, including myelination, epilepsy, memory, mood and degenerative diseases (Birzniece et al., 2006; El Etr et al., 2005; Scharfman and MacLusky, 2006; Vallee et al., 2001). PROG also has therapeutic potential as a neuroprotective substance after brain injury, and is an important agent for hormone replacement therapy (Genazzani et al., 2005; Schumacher et al., 2007b; Stein, 2005, 2008).

Orally administered PROG has low bioavailability due to its high first-pass metabolism in the gastrointestinal tract and liver (de Ziegler and Fanchin, 2000). With the aim of overcoming this restriction in using PROG therapeutically, alternative routes of administration have been investigated, including the transdermal, i.m., intravaginal, and intranasal routes (Goletiani et al., 2007). The intranasal route would seem to be advantageous for PROG administration, since it is non-invasive, offers the possibility of bypassing the first-pass metabolism, having a rapid onset of drug action, and potentially providing a direct delivery of the drug to the CNS (Costantino et al., 2007).

The finding that PROG can modulate the activity of $GABA_A$, NMDA, nACh and σ1 receptors, indicates that the activity of several neurotransmitter systems in the brain

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Abbreviations: BL-AM, basolateral amygdala; DA, dopamine; DOPAC, 3,4-dihydroxyphenyl acetic acid; EDTA, ethylenediaminetetraacetic acid; HVA, homovanillic acid; nACh, nicotinic acetylcholine; NMDA, *N*-methyl-D-aspartate; NS, neostriatum; PROG, progesterone.

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can be influenced by its action. There is evidence for an influence on the mesolimbic and mesocortical dopaminergic systems and reward-associated behavior by gonadal steroid hormones in humans, implicating an impact on vulnerability to drug abuse, neuropsychiatric diseases with differential expression across males and females, and hormonally mediated mood disorders (Dreher et al., 2007). It has been demonstrated that systemic administration of PROG increases dopamine (DA) release in the striatum of intact male and ovariectomized female rats (Petitclerc et al., 1995). Also, systemic PROG administration potentiated the increase in mesocortical dopaminergic neurons activity induced by ethanol (Dazzi et al., 2002). However, to our knowledge, there is, at present, no information on how intranasally administered PROG can affect brain neuronal activity. Based on evidence for an involvement of the dopaminergic system in the effects of PROG, and on the recent finding that PROG activates the amygdala in humans (van Wingen et al., 2008), in the present study we investigated the effects of intranasally administered PROG on dopaminergic activity in the brain of male rats using microdialysis. For comparison purposes, we also assessed the effects of s.c. PROG administration on the same neurochemical parameters. Two brain areas were investigated: the basolateral amygdala (BL-AM, comprising the lateral, basolateral and basomedial nuclei) and the neostriatum (NS). We anticipated a higher effectiveness of intranasal administration of PROG in the present study based on results obtained in prior studies, comparing systemic with intranasal administration of DA There, the intranasal route was much more effective in enhancing striatal DA release than the systemic (de Souza Silva et al., in press).

EXPERIMENTAL PROCEDURES

Subjects

Adult male Wistar rats were purchased from the local breeding facility (Tierversuchsanlage, University of Düsseldorf, Düsseldorf, Germany). Thirty-three animals were used for intranasal PROG administration experiment (weight: 343.8±5.2 g, mean±S.E.M.), 29 for the s.c. PROG administration experiment (weight: 351.9±6.1 g, mean±S.E.M.). All animals were kept in standard Macrolon cages (Type IV) in groups of five animals per cage. Animals undergoing microdialysis experimentation were housed individually after implantation of guide-cannulae. They were maintained under a reversed 12-h light/dark cycle (lights on: 7:00 am) in a temperature- (21 °C) and humidity-controlled room with food and water available *ad libitum*. All experiments were carried out according to the German Law of Animal Protection of 1998 (in accord with the NIH Guide for the Care and Use of Laboratory Animals-NIH publications No. 80-23, revised 1996). Efforts were made to minimize the number of animals used and their suffering.

Drug

PROG (4-pregnene-3,20-dione, Sigma-Aldrich, St. Louis, MO, USA) was used. For intranasal administration, PROG was suspended in 10 μl of gel (composed of a viscous castor oil mixture, M et P Pharma AG, Stans, Switzerland), and injected into each nostril (5 μl each) employing a micropipette for viscous media (Transferpettor, Brand GMBH +CO KG, Wertheim, Germany). For s.c. injections, PROG was dissolved in sesame oil (Sigma-Aldrich) and injected in a volume of 1 ml/kg.

Surgery

Animals were deeply anesthetized with a mixture of ketamine hydrochloride (90.0 mg/kg, Ketavet, Pharmacia & Upjohn GmbH, Erlangen, Germany) and xylazine hydrochloride (8.0 mg/kg, Rompun, Bayer, Leverkusen, Germany) and fixed in a Kopf stereotactic frame. In each rat, two guide-cannulae with a thread on the top (15 mm, 22 G, stainless steel) were implanted, at the dorsal border of the BL-AM (AP: −3.0 mm, ML: ±5.0 mm, DV: −6.0 mm) and the NS (AP: 0.0 mm, ML: ±3.0 mm, DV: −3.0 mm). Each cannula was implanted in the left or right BL-AM or NS, alternately, so that each animal had one guide-cannula implanted in the left hemisphere, and the other in the right hemisphere. All coordinates were taken relative to bregma, according to the atlas of Paxinos and Watson (1986). They were fixed to the skull with two screws (stainless steel, d=1.4 mm) and dental cement. In the 24 h period after surgery the animals were given 100 μl Novalgin (containing 500 mg/ml metamizol-sodium, Aventis Pharma, Frankfurt, Germany) orally at 6 h intervals. Animals were allowed to recover for at least 6 days before beginning of microdialysis experimentation.

Microdialysis procedure

Experiments were conducted between 9:00 am and 6:00 pm. The animals were anesthetized with 1.25 g/kg urethane (Sigma-Aldrich) i.p. and placed on a heating pad in an acrylic box (45×25×22 cm). Body temperature was maintained during the experiment between 36.5–37.5 °C by a temperature controller (CMA 150, Carnegie, Sweden). A catheter was inserted into the i.p. cavity to allow supply of water and ions during the course of the experiment, without touching the animal. The dialysis probes (4.0 and 2.5 mm active membrane length for the NS and BL-AM, respectively) of a concentric design (for construction details see: Boix et al., 1995) were inserted through the guide-cannulae and fixed to the thread. Next, they were connected to syringes attached to a microdialysis pump (CMA 100) and perfused at a flow rate of 1.5 μl/min with artificial cerebrospinal fluid containing Na^+ 146 mM, K^+ 4 mM, Ca^{2+} 2.2 mM and Cl^- 156 mM. After a stabilization period of 2 h, the samples were collected every 30 min into vials containing 10 μl of 0.05 M $HClO_4$, in which 100 pg of deoxyepinephrine (internal standard) was dissolved. After four baseline samples were collected, one of the treatments was applied: For the intranasal administration experiment, PROG (0.5, 1.0 or 2.0 mg/kg) or its vehicle was applied; for the s.c. administration experiment, PROG (1.0, 2.0 or 4.0 mg/kg) or its vehicle was applied. Thereafter, sampling continued for additional 4 h.

Analysis

Immediately after collection, the samples were analyzed for the content of DA and its metabolites, 3,4-dihydroxyphenyl acetic acid (DOPAC) and homovanillic acid (HVA), using high performance liquid chromatography. For the separation we used a 125×2 mm reversed-phase column (120-5 C18, Macherey Nagel, Düren, Germany), perfused with a mobile phase, composed of 99.5 mM chloroacetic acid, 0.53 mM sodium octyl sulfate, 0.5 mM EDTA, 4 mM KCl, 6% v/v acetonitrile and 0.8% v/v tetrahydrofuran, with pH adjusted to 3.25 using 6 M NaOH solution. Quantification was performed by amperometric detection (Intro, Antec, Leyden, Netherlands) with the potential set at +530 mV vs. an ISAAC reference electrode (Antec) at 28 °C. The limit of detection was 0.5 pg per sample for DA and pg per sample for the metabolites at a signal-to-noise ratio of 2:1.