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Modulation of ventral pallidal dopamine and glutamate release by the intravenous anesthetic propofol studied by *in vivo* microdialysis

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Summary. The intravenous anesthetic propofol is reported to have various psychological side effects as hallucinations, sexual disinhibition, or euphoria. Hedonic and rewarding states like these are modulated by the dopaminergic system in the nucleus accumbens, prefrontal cortex and also in the ventral pallidum and by the glutamatergic system in the neocortex and limbic system. In the present study, propofol was administered either alone or in combination with the GABAA receptor antagonist bicuculline via reverse microdialysis into the ventral pallidum of freely moving rats. Dialysis fractions were taken every 20 min and analyzed for dopamine and glutamate using high performance liquid chromatography. Application of propofol decreased dopamine levels in the ventral pallidum. This effect seems to be mainly mediated through GABAA receptors, since it was compensated by the GABAA receptor antagonist bicuculline. Propofol and propofol plus bicuculline exerted no effect on glutamate release in this brain region. The reduced dopamine release in ventral pallidum was most probably mediated through a GABAergic feedback loop from the ventral pallidum via the nucleus accumbens to the dopaminergic neurons of the ventral tegmental area or by long loop feedback. As an increase but not a decrease of dopamine release in the ventral pallidum is involved in hedonic and rewarding properties, similar symptoms induced by propofol seem to be unrelated to an action of propofol in the ventral pallidum.

Keywords: Propofol – Dopamine – Glutamate – Ventral pallidum – Bicuculline

Introduction

The phenol derivative propofol (2,6-diisopropylphenol) is widely used as an intravenous anesthetic because of its short recovery time (Peduto et al., 1991). Regarding the mechanism of action, several experimental studies have shown that propofol possesses GABA-mimetic (Hara et al., 1993; Hales and Lambert, 1991) and GABA-potentiating properties (Hales and Lambert, 1991; Hara et al., 1994) *in vitro*. *In vivo*, propofol exerts inhibitory effects on nondopaminergic neurons in the pars reticulata of the substantia nigra via strionigral GABAergic neurons (Peduto et al., 1991).

In addition to the anesthetic properties of propofol, various hedonic and rewarding effects such as hallucinations, sexual disinhibition, or euphoria have been reported during recovery from anesthesia (Hunter et al., 1987; Briker, 1988). The most frequent side effect reported by patients is a feeling of well-being (Brazzalotto, 1989). Even more, propofol has been found to induce pleasant mood-changes in patients at subanesthetic doses (Borgeat et al., 1994). Only few clinical studies have examined the putative effect of propofol on affective states (Zacny et al., 1993; Zacny et al., 1992; Whitehead et al., 1994) and the question whether propofol has a pleasant effect is still a matter of debate (Sneyd, 1994). Also in rats, propofol elicits a pleasant affective state and acts anxiolytic (Pain et al., 1997, 1999).

The ventral pallidum (VP) is the output structure of the nucleus accumbens in the ventral corticostriatothalamocortical loop. Information processing in this loop is critically involved in motor behavior and reinforcement (Kretschmer, 2000a). Association of reinforcement or drug reward with the mesolimbic dopamine system is well established (Kretschmer, 2000b). However, apart from the dopaminergic system also the glutamatergic system seems to be implicated in reward functions (Bardo, 1998).

Therefore, the present study investigated modulation of dopaminergic and glutamatergic release in the VP by local administration of propofol.

Materials and methods

Chemicals

Propofol was obtained from RBI (Cologne, Germany). All other chemicals were purchased from Sigma (Deisenhofen, Germany). Propofol was dissolved in dimethyl sulfoxide (DMSO) in a concentration of 10 mM and diluted to the appropriate concentration in the artificial cerebrospinal fluid (aCSF, composition see below) on the day of experiment. The pH of the final drug solution was adjusted to 7.4.

Subjects

Male Sprague Dawley rats (Charles River, Sulzfeld, Germany) weighing 220–260 g were housed in groups of 6–8 in standard laboratory cages under constant conditions of temperature (23 ± 2°C) and light (light from 6 a.m. to 6 p.m.). They were fed once a day with standard laboratory chow (12 g/day/rat). Tap water was available ad libitum. All animal procedures adhered strictly to the national ethical guidelines regarding the care and use of animals, and were approved by the local council of animal care (ZO1/96; Tübingen, Germany). The "principles of laboratory animal care" (NIH publication No. 86-23, revised 1985) were followed.

Animal procedures

Guide cannulas (CMA microdialysis; Semrau AG, Sprockhövel, Germany) were unilaterally implanted under deep anesthesia (chloral hydrate 350 mg/kg i.p.) into the VP (AP − 0.3 mm, L + 2.5 mm, V − 7.0 mm; according to Paxinos and Watson, 1986). Animals were allowed to recover from surgery for at least three days. On the day of experiment, microdialysis probes (active membrane length of 2 mm, CMA/12, CMA microdialysis) were slowly lowered through the guide cannulas. Rats were gently placed into an open field equipped with a freely moving setup (CMA microdialysis). Probes were perfused with aCSF (147 mM NaCl, 2.5 mM KCl, 1.3 mM $CaCl_2$ and 0.9 mM $MgCl_2$, adjusted to pH 7.4) at a flow rate of 3.2 µl/min. After an equilibration period of three hours, three 20 min fractions (0 min, 20 min, 40 min) were collected to determine baseline neurotransmitter release. Propofol (25 µM) was administered by reverse microdialysis from 40–100 min after the start of experiment, followed by a recovery period of 60 min. After the recovery period, propofol (25 µM) and the GABAA receptor antagonist bicuculline (100 µM) were coadministered via reversed microdialysis from 160–220 min followed by a second 60-min recovery period.

Behaviour was recorded and scored before and during the infusion period. Rats were killed by an overdose of pentobarbital at the end of experiment. Brains were removed and the localization of the probes was examined on coronal brain sections (30 µm) stained with cresyl violet.

Biochemical assays

Monoamines: Dialysis fractions (50 µl) were analyzed using reverse phase high performance liquid chromatography (HPLC) (Bischoff; Leonberg, Germany) with electrochemical detection (ESA; Bischoff). Dopamine, 3,4-dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA) were separated by a Nucleosil 5-C18 column (Bischoff) with a mobile phase containing 60 mM NaH_2PO_4, 0.1 mM EDTA, 0.2 mM octanesulfonic acid and 10% methanol (pH 3.73). Electrochemical detection was performed with a two-electrode system (ESA Coulochem 5100, Bischoff), with +320 mV at the first electrode and −250 mV at the second electrode. A detection limit of 0.1 nM was routinely achieved. The retention times of DA, DOPAC and HVA were 4.0 min, 5.8 min and 15.0 min, respectively.

Glutamate and aspartate: Dialysis fractions were analyzed using reverse phase HPLC with a F1000 fluorimetric detection (CMA/280 fluorescence detector; CMA microdialysis, Solna, Sweden) according to previous studies (Herrera-Marschitz et al., 1996) using pre-column derivatization with *o*-phtahldialdehyde/2-mercaptoethanol (OPA). 11 µl of the dialysate were mixed with 15 µL OPA, and left to react for 60 s at 4°C. Subsequently, the mixture was injected onto a Nucleosil-100 C18 5 µm particles column (Knauer, Berlin, Germany). Separation was achieved with a mobile phase containing 0.1 M sodium acetate, 6% methanol and 1.5% tetrahydrofuran (pH 6.95). Mobile phase was switched to 100% methanol for 2 min after each elution of glutamate and aspartate. The F1000 fluorimetric detector was set to a band-excitation wavelength of 370 nm and a band-detection wavelength of 450 nm. A detection limit of 10 nM was routinely achieved.

Statistics and data presentation

Basal neurotransmitter concentration was calculated as mean of three baseline samples. All neurotransmitter concentrations are expressed as a percentage of basal release. Data were calculated without correction for recovery values and are presented as mean ± SEM. Microdialysis data were analyzed by a repeated-measures one-way ANOVA followed by Fischer's LSD-(protected-t)-test when appropriate. The level of significance was set to $p \leq 0.05$.

Results

Modulation of ventral pallidal dopamine and glutamate release by propofol

The basal concentrations of dopamine was 0.80 ± 0.22 nM in the VP. Propofol (25 µM) was administered via reverse microdialysis from 40–100 min after the start of the experiment. Dopamine levels decreased immediately after start of injection of propofol with a minimum of 32.5 ± 10.7% of baseline after 80 min and reached baseline levels when propofol infusion was terminated ($n = 6$, $p < 0.05$) (Fig. 1).

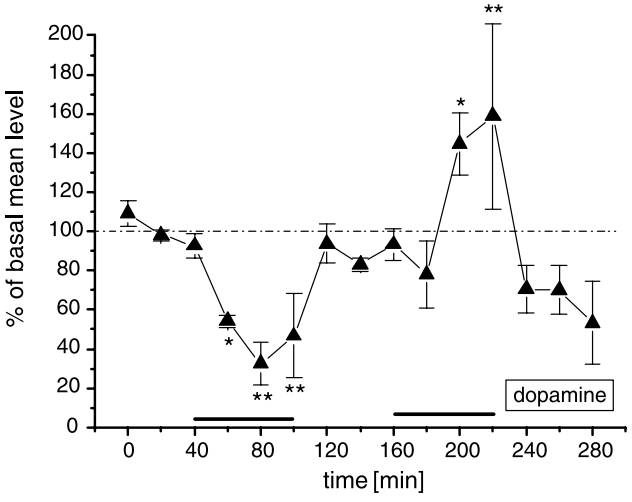

Fig. 1. Modulation of dopamine release in ventral pallidum of freely moving rats by propofol and bicuculline. All neurotransmitter concentrations are expressed as percentage of basal release. Basal neurotransmitter concentration was calculated as mean of three samples measured immediately after the start of the experiment (0 min, 20 min, 40 min). Local administration of propofol (25 µM) from 40–100 min by reverse microdialysis, coadministration of propofol (25 µM) and bicuculline (100 µM) from 160–220 min (bars). Data are presented as mean ± SEM of six experiments. Microdialysis data were analyzed by a repeated-measures one-way ANOVA followed by Fischer's LSD-(protected-t)-test (*$p < 0.05$; **$p < 0.01$)

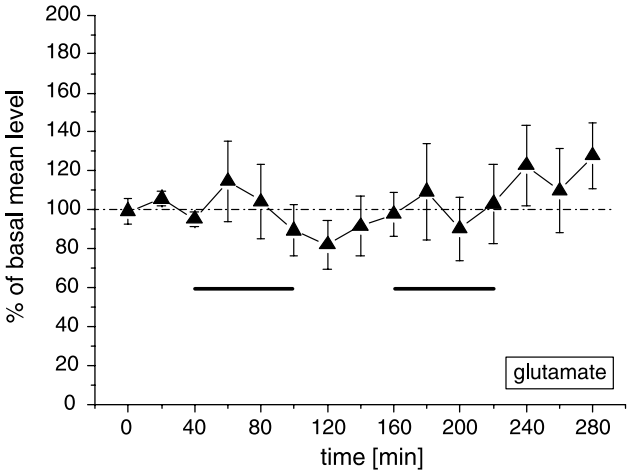

Fig. 2. Modulation of glutamate release in ventral pallidum of freely moving rats by propofol and bicuculline. All neurotransmitter concentrations are expressed as a percentage of basal release. Basal neurotransmitter concentration was calculated as mean of three samples measured immediately after the start of the experiment (0 min, 20 min, 40 min). Local administration of propofol (25 μM) from 40–100 min by reverse microdialysis, coadministration of propofol (25 μM) and bicuculline (100 μM) from 160–220 min (bars). Data are presented as mean $\pm$ SEM. SEM. Microdialysis data were analyzed by a repeated-measures one-way ANOVA followed by Fischer's LSD-(protected-t) test

Infusion of propofol exerted no significant effects on glutamate levels (Fig. 2). Propofol infusion had no effects on motor behavior (data not shown).

Effects of coadministration of propofol and bicuculline on ventral pallidal dopamine and glutamate release

Propofol (25 μM) plus the GABAA receptor antagonist bicuculline (100 μM) were administered via reverse microdialysis from 160–220 min. Dopamine levels increased and reached a maximum of $158.8 \pm 47.3\%$ at 200 min and decreased immediately after the end of drug infusion ($n = 6$, $p < 0.05$) (Fig. 1).

Coadministration of propofol and bicuculline had no significant effect on ventral pallidal glutamate levels (Fig. 2). Coinfusion of propofol + bicuculline increased motor activity of rats by an increase of stereotyped sniffing behavior and forward locomotion (data not shown).

Localization of microdialysis probes

After the end of experiment, rats were sacrificed and brains were removed. The localization of the probes was examined on coronal brain sections (30 μm) stained with cresyl violet. All implanted probes were localized in the VP (data not shown).

Discussion

The present study indicates that propofol (25 μM) decreased dopamine release in the VP that seems to be mediated by GABAA receptors. This finding is in contrast to behavioral studies and patient reports, suggesting a rewarding and by this way hedonic component of propofol (Pain et al., 1997; 1999; Hunter et al., 1987; Briker, 1988; Brazzalotto, 1989), if we take previous reports into account that reward is associated with an increase of dopamine in VP. Calculation of the local concentration of propofol in VP indicates an approximately concentration of 2.5 μM if we consider 10% recovery of the 25 μM propofol solution. This concentration is pharmacologically relevant, as an EC_{50} concentration of 0.4 μM can be calculated for general anesthesia in mammals (Franks and Lieb, 1994).

In the VP dopaminergic projections from the ventral tegmental area (VTA) are described. Stimulation of the dopaminergic terminals in the VP increases locomotor activity (Klitenick et al., 1992) and is positively correlated to rewarding properties, i.e. intracranial self-stimulation (McBride et al., 1999; Panagis and Spyraki, 1996). Various hedonic and rewarding effects of propofol such as hallucinations, sexual disinhibition or euphoria, have been reported in humans and animals (Hunter et al., 1987; Pain et al., 1996). However, our results showed a decrease in ventral pallidal dopamine release after local administration of propofol. Therefore, it can be concluded that a hedonic component of propofol is not correlated to a local action on dopamine release in VP. Since the selective GABAA receptor antagonist bicuculline (Chebib and Johnston, 1999) was able to compensate the action of propofol in this brain area, propofol seems to decrease dopamine release in VP via activation of GABAA receptors. An effect of GABAA receptors on dopamine release in the VP has previously been reported (Gong et al., 1998).

GABAA receptors are postsynaptically located (Wisden and Stephens, 1999), it therefore seems likely that propofol decreases dopamine levels through feedback or feedforward projections in or to the VP carrying GABAA receptors. For example, a short feedback loop exists between the VP and the dopaminergic VTA via the nucleus accumbens (Klintenick et al., 1992). Interestingly, propofol blocks also neuronal activity of substantia nigra dopamine neurons after i.v. application, showing that propofol blocks dopaminergic activity also via the systemic route. Nevertheless, in the substantia nigra the effect seems to be mediated by GABAB receptors (Schwieler et al., 2003).

Apart from the dopaminergic input from the VTA, the VP also receives glutamatergic input from cortical and limbic areas (Kretschmer et al., 2000; Kretschmer, 2000a). The glutamatergic system in the VP seems also to be implicated in reward functions (Bardo, 1998). However, neither application of propofol nor coadministration of propofol and bicuculline exerted any significant effect on glutamate release in the VP. This further supports the finding that the VP does not mediate hedonic effects exerted by propofol. Other structures like the nucleus accumbens may primarily be involved.

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