ELSEVIER

European Journal of Pharmacology 398 (2000) 247–250

ejp

www.elsevier.nl/locate/ejphar

Short communication

Effects of ensaculin on dopamine metabolite levels and K^+-induced glutamate release

Peter Teismann, Boris Ferger *

Institute of Pharmacology and Toxicology, Faculty of Pharmacy, University of Marburg, Ketzerbach 63, D-35032 Marburg Germany

Received 14 February 2000; received in revised form 4 April 2000; accepted 7 April 2000

Abstract

In vivo microdialysis with the new antidementia compound ensaculin was performed in freely moving rats to study the alterations in dopaminergic and glutamatergic neurotransmission. Ensaculin (0.1 and 1 mg/kg i.p.) significantly increased extracellular levels of the dopamine metabolites 3,4-dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA). Furthermore, ensaculin (1 mg/kg i.p.) showed a non-significant tendency to reduce the K^+-induced glutamate release. The data suggest that ensaculin may have moderate D_2 antagonistic properties. Thus, besides its possible role as a cognitive enhancer, ensaculin may also have moderate antipsychotic properties.

Keywords: Ensaculin; Dopamine; DOPAC (3,4-dihydroxyphenylacetic acid); Glutamate; Microdialysis; Rat

1. Introduction

In Alzheimer's disease, a deficiency in the cholinergic neuronal transmission is evident, shown by the loss of choline acetyltransferase and other cholinergic markers (Finch and Roth, 1999). Besides the improvement of cholinergic function, other approaches, such as altering the dopaminergic or serotonergic neurotransmission, could be promising strategies to treat Alzheimer's disease and related cognitive disorders (Nöldner et al., 1996). As glutamate levels (Pomara et al., 1992) and glutamine synthetase (Tumani et al., 1999) are increased in patients with Alzheimer's disease, a modulation of the glutamatergic system could be a useful approach as well.

Structure–activity studies on scoparon derivatives led to the identification of ensaculin (see Fig. 1) as a potential antidementia agent for the treatment of Alzheimer's disease and related cognitive disorders. Ensaculin was designed as a cognitive enhancer and is currently undergoing clinical phase II trials for Alzheimer's disease. Ensaculin showed acetylcholine esterase inhibitory properties in vitro (Nöldner et al., 1996) and a unique combination of modulatory effects on dopaminergic (D_2)-, serotonergic (5-HT_{1A})-, and adrenergic (α_1) neurotransmitter systems was observed. Additionally, ensaculin is described as a functional *N*-methyl-D-aspartate (NMDA) receptor antagonist (Lishko et al., 1998).

Enhanced learning facilities after the treatment with ensaculin were shown by Klusa et al. (1994) in the passive avoidance test in mice and rats and in the conditioned avoidance test in rats. These findings lead to the conclusion that the pharmacological properties of ensaculin are those of a novel neuronal activator (Nöldner et al., 1996).

We used the microdialysis technique to study alterations in dopaminergic and glutamatergic neurotransmission after administration of ensaculin in rat.

2. Materials and methods

2.1. Animals

Adult male albino Wistar rats (Hsd/Cpb:WU, Fa. Harlan–Winkelmann Borchen, Germany), weighing about 300 g at the time of implantation, were used in this study. The ambient room temperature was maintained at 23 ± 2°C and

* Corresponding author. Tel.: +49-6421-2825834; fax: +49-6421-2828918.

E-mail address: ferger@mailer.uni-marburg.de (B. Ferger).

0014-2999/00/$ - see front matter
PII: S0014-2999(00)00290-9

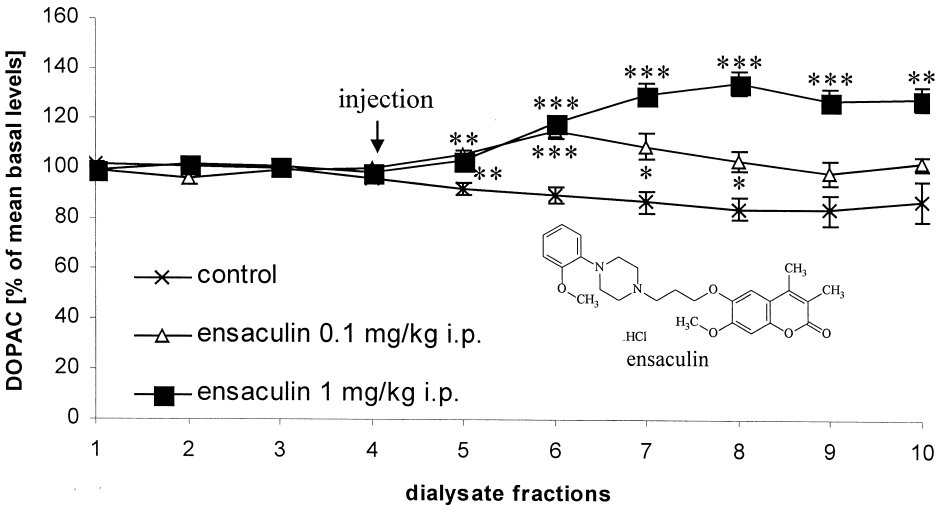

Fig. 1. Effect of ensaculin (0.1 mg/kg (△) or 1 mg/kg i.p. (■)) or saline (X) on extracellular concentration of DOPAC in striatal dialysates. Abscissa: time before and after the administration of ensaculin or saline; ordinate: extracellular levels of DOPAC in percentage of mean basal levels. The mean of the pre-drug dialysates was regarded as 100% basal levels (dialysate fractions 1–4). Data are mean ± S.E.M. ($n = 6$–13). $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ by ANOVA with post hoc *t*-test compared with control animals.

the relative humidity at 55 ± 5%. The animals were kept on a 12-h light–dark cycle (lights on from 0700 to 1900 h) and were housed individually in Plexiglas cages (40 × 20 × 24 cm) and fed on standard food (Altromin®) and tap water.

2.2. Microdialysis experiments

Five days before the experiment, a guide cannula was implanted aiming at the striatum under chloral hydrate anaesthesia (400 mg/kg i.p.) (co-ordinates from bregma: *A*: +1.25, *L*: +2.6, *V*: −2.5 according to the atlas of Paxinos and Watson, 1986). The guide cannula was fixed to the skull with stainless steel screws and methylacrylic cement. On the day of microdialysis experiment, the rats were placed in a system for freely moving animals. After removal of the guide obturator, the microdialysis probes (CMA 12; Carnegie Medicin, Stockholm, Sweden; membrane diameter 0.5 mm; membrane length 4 mm) were introduced and perfused with a modified Ringer's solution (Na^+ 147 mM, K^+ 4 mM, Ca^{2+} 1.3 mM, Mg^{2+} 1 mM, Cl^- 155.6 mM dissolved in bidistilled water) at a flow rate of 2 μl/min. Microdialysis experiments started with a 180 min equilibration period. Afterwards, four 20 min fractions were collected for the calculation of basal neurotransmitter levels, which were set at 100% (dialysate fractions 1–4). Then, ensaculin (0.1 or 1 mg/kg) or saline was administered intraperitoneally and the alterations of dopamine and its metabolites were measured for 120 min (dialysate fractions 5–10).

In another set of experiments for depolarization-induced glutamate release, the K^+ concentration in the perfusion fluid was raised to 100 mM for 40 min (dialysate fractions 5 + 6). The Na^+ concentration in the perfusion liquid was reduced correspondingly. Afterwards, the perfusion fluid was switched back to the pre-stimulation conditions and measurement was continued for further 120 min (dialysate fractions 7–12). Ensaculin (1 mg/kg i.p.) or saline was administered 40 min before the onset of K^+-induced glutamate release (dialysate fractions 3 + 4).

2.3. High-performance liquid chromatography (HPLC) determination of dopamine, DOPAC and HVA

Samples were collected in vials containing 10 μl of 0.4 M perchloric acid and were analyzed by HPLC with a two-channel electrochemical detector (BAS LC 4C; Bioanalytical Systems, West Lafayette, IN, USA). A reversed-phase column (125 × 3.0-mm vertex column with precolumn) filled with Nucleosil 120-3 C18 (Machery-Nagel, Düren, Germany) was used for separation. The detector potential was set at 750 mV vs. an Ag/AgCl reference electrode with a range of 1–10 nA. The mobile phase contained 140 mg of octane sulfonic acid sodium salt, 100 mg of disodium EDTA and 6 ml of triethylamine in HPLC grade water. Phosphoric acid was used to adjust the pH to 2.9. After filtration (pore size 0.45 μm), 35 ml of acetonitrile was added to a final volume of 1 l. The mobile phase was degassed by an online degasser (CMA 260; Carnegie Medicin) and delivered by a high pressure pump at a rate of 0.5 ml/min. Data were recorded and analyzed by the HPLC computer system Gynkosoft (Gynkotek, Germering, Germany). The peak areas were integrated and calculated by means of an external standard calibration. The values of extracellular neurotransmitter levels and metabolites are expressed as a percentage of the basal levels and are not corrected for the recoveries of the probes, which were approximately 15%.

2.4. HPLC determination of glutamate

Samples were collected every 20 min and were analyzed for glutamate after pre-column derivatisation with *o*-phthaldialdehyde with HPLC and fluorescence detection (Fluorescence HPLC Monitor RF 535, Shimadzu, Kyoto, Japan). Each liter of the mobile phase contained 1.03 g boric acid and 13.61 g sodium acetate in Millipore-filtered water. After filtration (pore size 0.45 μm), 200 ml methanol were added to a final volume of 1 l. The mobile phase was delivered by a high-pressure pump at a rate of 0.4 ml/min and degassed by an online degasser (CMA 260, Carnegie Medicin). Data were recorded and analyzed by the HPLC computer system Gynkosoft (Gynkotek). The areas of glutamate peaks were integrated and calculated by means of an external standard calibration.

2.5. Drugs and chemicals

Ensaculin (KA-672.HCl) was a generous gift from Dr. Willmar Schwabe, Germany. Dopamine HCl, 3,4-dihydroxyphenylacetic acid (DOPAC), homovanillic acid (HVA), glutamate, sodium acetate, boric acid, *o*-phthaldialdehyde and 1-octane sulfonic acid sodium salt were obtained from Sigma Chemie, Munich, Germany. HPLC grade acetonitrile and methanol were purchased from Merck, Darmstadt, Germany. All the standards were of the highest purity commercially available. The standards for HPLC were dissolved in 0.1 M perchloric acid. Ensaculin was dissolved in saline. Ensaculin and saline were administered intraperitoneally in a volume of 10 ml/kg body weight.

3. Results

Basal DOPAC levels (mean of dialysates 1–4) of the saline and ensaculin (0.1 and 1 mg/kg) treated groups were set at 100% and values were 1145.8 ± 13.5, 1115.7 ± 96.8 and 1127.3 ± 12.3 nmol/l (mean ± S.E.M.), respectively.

After injection of ensaculin (0.1 mg/kg), we found a 1.15-fold increase of the DOPAC level. The injection of ensaculin (1 mg/kg) produced a 1.3-fold increase of DOPAC levels. This increase was significant in the first dialysate after ensaculin administration and persisted for 80 min after the lower dose, and for 100 min after the injection of the higher dose of ensaculin (Fig. 1). Furthermore, a 1.1-fold increase in striatal HVA level was observed after the injection of ensaculin (0.1 mg/kg). The injection of ensaculin (1 mg/kg) produced a 1.36-fold increase of striatal HVA levels. This increase was significant in the second dialysate after ensaculin (1 mg/kg) administration and persisted for 80 min. After the injection of ensaculin (0.1 mg/kg), the increase of HVA level was significant in the first dialysate, 20 min after injection, and persisted for a further 20 min.

Dopamine levels were significantly increased 60 min after treatment with 1 mg/kg ensaculin. In the saline and ensaculin (0.1 mg/kg) treated groups, no significant changes compared with the basal levels were observed during the experiment.

The mean glutamate levels of the two pre-drug dialysate fractions of the saline and ensaculin (1 mg/kg) group were regarded as 100% basal levels. Basal glutamate levels were not significantly altered by ensaculin (1 mg/kg i.p.),

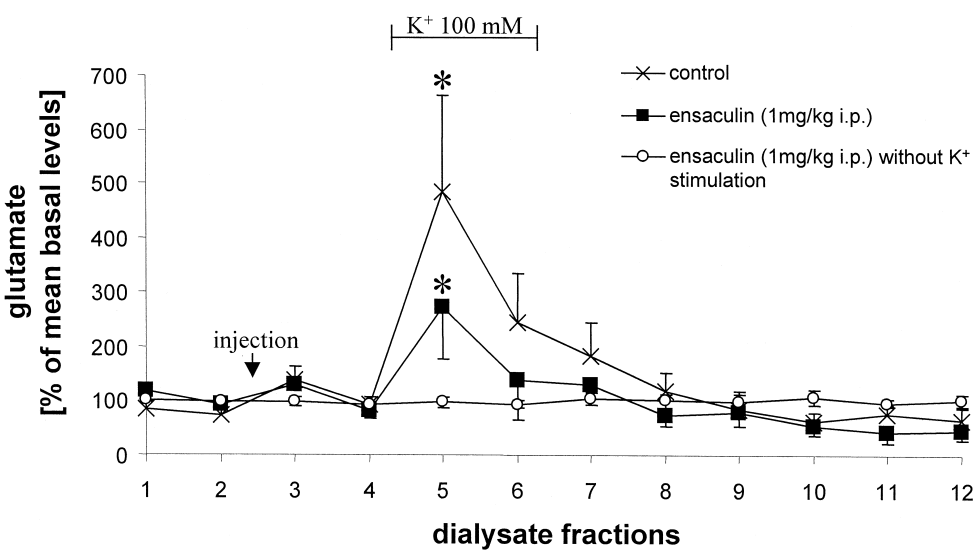

Fig. 2. Time course of the extracellular glutamate concentration, expressed as percentage of basal levels: effect of ensaculin (1 mg/kg i.p.; ■) and saline (X) on the K^+-induced overflow of glutamate. The open circles (○) show the effect of ensaculin (1 mg/kg i.p.) on glutamate levels without K^+ stimulation. The mean of the pre-drug dialysates were taken as 100% basal levels (dialysate fractions 1 + 2). Data are mean ± S.E.M. ($n = 6$). $^*P < 0.05$ by ANOVA with post hoc *t*-test compared with control animals.

which were 0.5 ± 0.1 and 0.51 ± 0.11 μmol/l, respectively. During the stimulation with high K^+ concentration, the glutamate levels increased 4.1-fold in the saline-treated group (Fig. 2). The treatment with ensaculin led to a 2.8-fold elevation of K^+-induced increase of glutamate, which was not significantly different, compared with the saline-treated group (Fig. 2).

4. Discussion

In the present microdialysis study, dopamine levels after the administration of ensaculin (1 mg/kg) were only significantly increased 60 min after ensaculin injection, whereas the lower dose of ensaculin produced no significant changes. In contrast, the metabolites of dopamine, namely DOPAC and HVA, were significantly increased in a dose-dependent manner directly after the injection up to the end of the experiment. Ungerstedt (1984) found similar results for DOPAC and HVA in a microdialysis study in the striatum of rats after injection of the well-known dopamine D_2 receptor antagonist haloperidol. Unexpectedly, dopamine was not increased due to haloperidol or as in the present study, ensaculin injection, although the blockade of presynaptic dopamine D_2 receptors are believed to increase extracellular dopamine levels (Ungerstedt, 1984). However, antipsychotics increase impulse flow in the nigrostriatal pathway and also increase striatal DOPAC, whereas drugs that block or decrease impulse flow reduce DOPAC levels (Cooper et al., 1996). Behavioral, electrophysiological and receptor binding studies of ensaculin reveal the involvement of the dopamine D_2 receptor antagonistic, as well as serotonin 5-HT_{1A} receptor agonistic properties (Nöldner et al., 1996, Winter et al., 1998).

The pharmacological profile of ensaculin is not completely elucidated and in vivo studies are rare. This is the first in vivo microdialysis study to investigate the effects of ensaculin on dopminergic and glutamatergic neurotransmission. However, significant alterations were evident only in dopaminergic neurotransmission. The significant ensaculin-induced increase in the extracellular dopamine metabolites in the present study are in line with binding studies which showed high affinity to dopamine D_2 receptors in the nanomolar range (Nöldner et al., 1996) and in agreement with behavioral experiments. Ensaculin enhanced the haloperidol-induced catalepsy, but was not cataleptic by its own (Nöldner et al., 1996).

Ensaculin might be useful as a moderate antipsychotic drug, without producing unwanted motor side-effects as caused by haloperidol.

The ongoing clinical phase II trial of ensaculin will show if the described pharmacological profile of ensaculin is useful for the treatment of patients with dementia accompanied with psychiatric disorders.

Acknowledgements

This study was supported by a grant of the Deutsche Forschungsgemeinschaft (Fe 465/1-3). We thank Mrs. Andrea Lehr for the linguistic revision of the manuscript and Dr. S. S. Chatterjee and Dr. M. Nöldner (Dr. Willmar Schwabe, Karlsruhe, Germany) for the helpful discussion, as well as for providing us with ensaculin. The experimental protocols were approved by the appropriate institutional governmental agency (Regierungspräsident Gießen, Germany).

References

Cooper, J.R., Bloom, F.E., Roth, R.H., 1996. The Biochemical Basis of Neuropharmacology. 7th edn. Oxford Univ. Press, New York, NY.

Finch, C.E., Roth, G.S., 1999. Biochemistry of aging. In: Siegel, G.J., Agranoff, B.W., Albers, R.W., Fisher, S.K., Uhler, M.D. (Eds.), Basic Neurochemistry. 7th edn. Raven Press, New York, pp. 613–633.

Klusa, V., Germane, S., Chatterjee, S.S., Nöldner, M., 1994. KA-672.HCl: a new orally active antidementia agent. Eur. J. Pharm. Sci. 2, 19, Abst.

Lishko, P.V., Maximyuk, O.P., Chatterjee, S.S., Nöldner, M., Krishtal, O.A., 1998. The putative cognitive enhancer KA-672.HCl is an uncompetitive voltage dependent NMDA receptor antagonist. NeuroReport 9, 4193–4197.

Nöldner, H., Hauer, H., Chatterjee, S.S., 1996. Anseculin hydrochloride. Drugs Future 21, 779–781.

Paxinos, G., Watson, C., 1986. The Rat Brain in Stereotaxic Coordinates. 2nd edn. Academic Press, London.

Pomara, N., Singh, R., Deptula, D., Chou, J.C., Schwartz, M.B., LeWitt, P.A., 1992. Glutamate and other CSF amino acids in Alzheimer's disease. Am. J. Psychiatry 149, 251–254.

Tumani, H., Shen, G., Peter, J.B., Bruck, W., 1999. Glutamine synthetase in cerebrospinal fluid, serum, and brain: a diagnostic marker for Alzheimer disease?. Arch. Neurol. 56, 1241–1246.

Ungerstedt, U., 1984. Measurement of neurotransmitter release by intracranial dialysis. In: Marsden, C.A (Ed.), Measurement of Neurotransmitter Release in Vivo. Wiley, Chichester, pp. 81–105.

Winter, J.C., Helsley, S.E., Rabin, R.A., 1998. The discriminative stimulus effects of KA 672, a putative cognitive enhancer: evidence for a 5-HT_{1A} component. Pharmacol. Biochem. Behav. 60, 703–707.