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EEG effect of basal forebrain neuropeptide Y administration in urethane anaesthetized rats

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Abstract

Neuropeptide Y (NPY) is present both in local neurons as well as in fibers in the basal forebrain (BF), an area that plays an important role in the regulation of cortical activation. In previous studies, NPY axons were found to innervate corticopetal cholinergic cells in this area. In addition, identified NPY positive neurons have been shown to be silent during cortical activation, but active during slow EEG waves. However, no in vivo studies have shown the effect of local NPY release in the BF on the EEG. In the present experiments, the EEG was examined following NPY injection (0.5 μl, 300–500 pmol) into the BF of urethane-anaesthetized rats. Fronto-parietal EEG was recorded on both sides and relative EEG power was calculated in the delta (0–3 Hz), theta (3–9 Hz), alpha (9–16 Hz) and beta (16–48 Hz) frequency bands. We found a significant increase in relative delta power and a decrease in the power of all higher frequency bands (theta, alpha, beta) after NPY injection. These results suggest that NPY can inhibit cortical activation via the BF.

Keywords: Neuropeptide Y; EEG; Basal forebrain; Cholinergic cells; Cortical activation

1. Introduction

Neuropeptide Y (NPY) is one of the most ubiquitous neuropeptides in the mammalian brain [2]. NPY is involved in several physiological functions such as feeding [9], memory [18], regulation of blood pressure [13], circadian rhythms [23], and possibly sleep–wake regulation. NPY is present in the basal forebrain (BF), an area that plays an important role in the regulation of cortical activation (for a recent review, see [25]). The BF contains a heterogeneous population of cholinergic and non-cholinergic (GABAergic, peptidergic, and glutamatergic) corticopetal neurons as well as various types of interneurons containing different peptides, including NPY and somatostatin [24].

The role of the NPY containing neurons in the modulation of sleep–wake states and cortical EEG via BF is not fully understood. NPY was found to colocalize with GABA in many forebrain neurons [1]. Single NPY neurons can innervate several cholinergic corticopetal neurons in the horizontal limb of the diagonal band [21,24] and in the substantia innominata [24]. NPY containing terminals were shown to form symmetrical synapses with cholinergic neurons [24] and are assumed to be inhibitory [19]. In anesthetized rats, NPY containing BF neurons represent a subpopulation of S-cells (slow-wave-active cells) [3] which are silent during spontaneous or tail pinch-induced cortical activation, but have a higher firing rate during episodes of cortical slow waves. These neurons have been suggested to inhibit cortically projecting cholinergic neurons [6]. The aim of the present experiments was to examine the effect of local injection of NPY into the BF by monitoring EEG changes in urethane-anesthetized rats.

2. Materials and methods

Adult male Wistar rats ($n = 9$), weighing between 280 and 400 g at the time of the experiment were anesthetized with

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urethane (1.0–1.2 g/kg, i.p.) and fixed in a stereotaxic frame (David Kopf) with Bregma and Lambda leveled. Rectal temperature was maintained at 37 °C with a heating pad attached to a thermostatic instrument. Experiments were carried out in accordance with the European Communities Council Directive and under the guidelines of the local Animal Care and Use Committee. For BF infusions, cannulas with a tip diameter of 30–50 μm were constructed from borosilicate glass capillaries (outer diameter: 2 mm; World Precision Instruments, Sarasota, FL) on a Sutter P-97 horizontal pipette puller (Sutter Instrument, CA, USA). Pressure injections (volume 0.5 μl, speed 0.25 μl/min) were carried out unilaterally with a microprocessor controlled syringe pump (IITC Inc., CA, USA) holding two Hamilton syringes. Each rat received one saline and one NPY injection in random order. At least 1.5–2 h elapsed between the two injections. NPY (Sigma-Aldrich, Schnelldorf, Germany) was dissolved in sterile physiological saline and administered in a dose of either 300 pmol/0.5 μl or 500 pmol/0.5 μl. Injections were made when anesthesia was superficial, and EEG was characterized by dynamic shifts between delta waves, spindles, and low-voltage fast activity (pattern I, see [4]). Special attention was paid to maintain the animals at an appropriate level of surgical anaesthesia throughout the experimental procedure. We never observed any movement in response to tail pinches.

EEG was recorded with screw electrodes from the frontal (A: Br 2.0 mm, L 2.0 mm) and parietal (A: Br-4.5 mm, L 2.0 mm) cortices on both sides. EEG signals were filtered (0.3–500 Hz) and amplified (10,000×) by an extracellular amplifier (A-M Systems). Signals were sampled at 200 Hz by a data acquisition system (DataWave Discovery) and the data was stored on hard disk for offline analysis.

Analysis was performed using custom-written software. EEG power spectra were constructed for consecutive 5 s epochs using Fast Fourier Transformation algorithm and averaged for 1 min long periods. From the power spectra, integrated relative power of the following frequency bands was calculated: delta (0–3 Hz), theta (3–9 Hz), alpha (9–16 Hz), beta (16–48 Hz), and the ratio between these power values on the treated and control side were determined.

Data points recorded during injections and tail pinches were excluded from the analysis as well as those points acquired during the 1 min period following tail pinches. EEG recordings lasted for 1 h (15 min control, 2 min injection, 43 min post-injection). Tail pinches were applied manually at 5, 10, 25, 35, 45, 55 min to test if EEG responses could be induced to noxious stimuli on both the treated and control side. It was applied for a few seconds until a clear cortical activation appeared in the control side. Ratio of EEG power values were normalized with the average of the control bins in the first 5 min before the first tail pinch. Statistical analyses (two-way ANOVA with cofactors: treatment and time followed by Newman-Keuls post-hoc tests) were carried out on the logarithmic values of the normalized ratios for consecutive 10 min periods using STATISTICA for Windows (StatSoft Inc.).

At the end of the recordings, animals were perfused transcardially with saline followed by 4% paraformaldehyde. Brains were removed and cryoprotected in the same fixative containing 30% sucrose until equilibration. Coronal sections (50 μm) were cut with a freezing microtome. Sections were stained with gallocyanine, dehydrated, and coverslipped with DepEx (Serva, Heidelberg, Germany). Injection sites were located based on the stereotaxic atlas of Paxinos and Watson [17].

To assess the spread of injected peptides in the BF, horseradish peroxidase (HRP; Sigma-Aldrich, Schnelldorf, Germany) was injected (5% solution, 0.5 μl) into the substantia innominata of two rats. Rats were perfused immediately after the HRP injection to prevent uptake and cellular transport. The peroxidase reaction was visualized with 3,3′-diaminobenzidine (DAB; Sigma-Aldrich, Schnelldorf, Germany) as the chromogen. The spread of HRP was found to be about 1000 μm from the injection site.

3. Results

For every recording session, a 10 min EEG sample was taken after burr holes had been drilled for the drug injection, but before the cannula was lowered. In most cases, a slight EEG asymmetry was seen between the two hemispheres. There was no obvious preference in EEG amplitude to either side; thus the asymmetry was probably due to small differences in electrode placement. Insertion of the cannula caused a more pronounced asymmetry and EEG amplitude was depressed on the cannulated side. However, this was only a transient phenomenon lasting for 15–20 min. Recordings started when EEG amplitude returned to the original level.

NPY administration at both doses (300 and 500 pmol/0.5 μl) caused significant changes in the ratio of EEG power values from the two hemispheres in every frequency range examined. No systematic dose effect was seen. Therefore, data obtained with the two doses were pooled. NPY injection increased relative EEG power on the injection side in the delta (0–3 Hz), and decreased in the theta (3–9 Hz), alpha (9–16 Hz), and beta (16–48 Hz) bands (Fig. 1). In all frequency bands, the effect continuously increased in the subsequent 10 min periods, peaked at 20–30 min following the injection of the peptide, then declined in the last period of the recording. The strongest changes were in the beta range and were highly significant already in the first 10 min period.

NPY was injected unilaterally and the opposite hemisphere served as control. In addition, we also tested whether vehicle injection itself was able to induce EEG changes. A cannula was lowered into the contralateral BF either before or after recording the NPY effect to examine the effect of physiological saline injection. Saline injections also affected the ratio of EEG power values calculated between the two sides, but these changes were much weaker