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Activation of tachykinin system in the pedunculopontine tegmental nucleus suppresses hippocampal theta rhythm in urethane-anesthetized rats

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Abstract. The pedunculopontine tegmental nucleus (PPN) is one of the reticular generators of the hippocampal theta rhythm. The PPN neuronal circuitry related to theta generation involves its cholinergic, GABA-ergic and glutamatergic components. Here we provide data indicating that the PPN tachykinin system may also be a part of this circuitry. In the experimental model of the tail-pinch elicited hippocampal theta in urethane-anesthetized rats (implanted with bilateral recording electrodes in the stratum moleculare of the upper blade of the dentate gyrus and with injection cannula unilaterally inserted into the PPN) it was found that intra-PPN microinjection of Substance P (SP) and [d-Pro2, d-Phe7, d-Trp9]-Substance P (DPDPDT) caused suppression of the theta and enhancement of the delta activity in the hippocampal EEG. Accordingly, there was approximately a 50% (SP) –70% (DPDPDT) decline of the peak power in the theta frequency range and a decrease by 0.4 Hz in the corresponding peak frequency (DPDPDT only) in both hippocampi. The circuitry through which SP exerts its effect in the PPN can be only hypothetical at present. We suggest SP-evoked activation (either direct or indirect through the glutamatergic inputs) of the GABA interneurons which may tonically inhibit PPN outputs to the other theta-relevant structures.

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INTRODUCTION

One of the dominant electroencephalographic events in the mammalian hippocampal formation is the theta rhythm, an oscillatory potential in the 3–12 Hz frequency range in rodents, which accompanies certain kinds of voluntary movements, attentive immobility and the paradoxical phase of sleep (REM) (Bland 1986, Monmaur et al. 1979, Sainsbury et al. 1987, Vanderwolf 1969, Vanderwolf et al 1977). This mode of hippocampal activity is linked, among others, to information processing and the formation of memory traces (Klimesch 1999, Tesche and Karhu 2000).

The hippocampal theta depends on inputs from pathways originating in the brainstem reticular formation, ascending *via* posterior hypothalamic and supramammillary nuclei to the medial septum/vertical limb of the diagonal band of Broca, which distributes them to limbic regions including the hippocampal formation (Bland 2000).

The pedunculopontine tegmental nucleus (PPN) in the pons is one of the theta-generating brainstem reticular nuclei, which was demonstrated in experiments where the stimulation of PPN cholinergic system by carbachol microinjection elicited the theta activity (Kinney et al. 1998, Vertes et al. 1993), while inactivation of all the PPN neurotransmitter systems by procaine microinjection suppressed the sensory-induced theta (Nowacka et al. 2002) in urethane anesthetized rats. The studies on the PPN involvement in the hippocampal theta generation so far have focused on three main PPN neurotransmitter systems: cholinergic, glutamatergic and GABAergic. And so, the activation of PPN muscarinic receptors evokes (Kinney et al. 1998, Vertes et al. 1993) while $GABA_A$ and NMDA receptors suppresses (Nowacka and Trojniar 2000) the theta in urethane anesthetized rats.

In rats, a number of neuromodulatory amines and peptides and/or their receptors were found in the PPN. Different groups of the PPN cholinergic neurons bear alpha 1 vs. alpha 2 adrenergic receptors, which are suggested to have different roles in sleep-wake state activities (Hou et al. 2002). The alpha 2 (autoreceptors) bearing group is supposed to be inhibited by noradrenaline while awake but disinhibited and maximally active during REM. Overlaps between the dopamine and orexin containing fibers extending from the hypothalamus were observed in the PPN (Baldo et al. 2003). The PPN neurons, some of them cholinergic, receive also serotonergic innervation form the dorsal raphe nucleus (Steininger et al. 1997). Concerning the neuromodulatory peptides, μ-opioid receptors, which contribute to opioid-induced REM inhibition within the pontine reticular formation, are present in the PPN (Capece et al. 1998) and μ receptors agonist, morphine, modulates the hippocampal theta elicited by intra-PPN administration of cholinomimetics in urethane anesthetized rats (Leszkowicz et al. 2004).

The tachykinin Substance P (SP) was found in the PPN, both in the cholinergic (mostly in the pars compacta, PPNc) (Kohlmeier et al. 2002, Standaert et al. 1986, Szeidemann et al. 1995) and non cholinergic (mostly in the pars dissipata, PPNd; Kohlmeier et al. 2002) neurons. The latter study has revealed that about 12% of cholinergic neurons in the PPNc and 3% in the PPNd contain SP, and about 67% of SP-positive neurons in the PPNc and 23% in the PPNd contain acetylcholine. Cholinergic PPN neurons coexpressing SP project to the pontine reticular nuclei involved in the generation of REM sleep (Kohlmeier et al. 2002), which in the rat is characterized by the presence of the hippocampal theta rhythm. Intravenous infusion of SP in men attenuates REM, which manifests as an increase in REM latency and a decrease in its density (Lieb et al. 2002). Besides REM sleep, another brain activity in which the hippocampal theta and SP effect meet is memory. The theta activity contributes to memory formation and retrieval (for review see Bastiaansen and Hagoort 2003) and SP is reported to have memory-promoting action (Hasenohrl et al. 2000). SP affects hippocampal formation directly in the CA1 region, where it increases the excitability of GABA-ergic interneurons thus inhibiting pyramidal cells (Ogier and Raggenbass 2003), which paces theta oscillations in the hippocampus (Banks et al. 2000, Chapman and Lacaille 1999).

The only report on the effect of SP on the hippocampal theta was presented by Kosinski and coauthors (1981, 1984) who used intracerebroventricular (*i.c.v.*) administration of C-terminal SP fragment (SP_{6-11}) and electrical stimulation of the midbrain reticular formation to evoke and then suppress the theta activity in rabbits. SP_{6-11} increased the number of pulse trains required to produce theta extinction (Kosinski et al. 1981) and had a dose-dependent facilitatory effect on the type 2 theta (4–7 Hz) (Kosinski et al. 1984), the one which is the subject of the present

study. Thus, *i.c.v.* administration of SP_{6-11} had a promoting effect on the theta.

Taken together, these studies suggest that the hippocampal field activity and tachykinins, in particular SP, can be functionally related. The present study addresses the question of a possible involvement of the PPN in the SP – theta relation. Since SP is present in the PPN on both cholinergic and non cholinergic neurons and the PPN influences the theta, it might be a level at which SP affects the hippocampal synchronization.

To test this, SP and its analogue [D-Pro^2, D-Phe^7, D-Trp^9]-Substance P (DPDPDT), described as SP antagonist/partial agonist (Bailey and Jordan 1984), were injected into the PPN while sensory induced hippocampal theta was recorded in urethane-anesthetized rats. Under urethane only the type 2 theta survives, the one which is cholinergically mediated and present during REM sleep and attentive immobility in the rat (Kramis et al. 1975, Sainsbury et al. 1987, Vanderwolf et al. 1977). Changes in the theta peak power and the corresponding peak frequency were used as a measure of the effect of intra-PPN injected substances.

METHODS

Subjects

Experiments were performed on 17 male Wistar rats, 4–6 months old, weighing about 300–500 g. The rats were housed in a vivarium, which was maintained in a 12-h light-dark cycle (lights on 6 A.M.) at 22 ± 1°C and provided with food and water *ad libitum*. The principles for the care and use of laboratory animals in research, as outlined by the Local Ethical Committee, were strictly followed and all the protocols were reviewed and approved by the Committee. All effort was made to minimize both animals' discomfort and the number of animals used.

Drugs

Substance P (SP) and its analogue, [D-Pro^2, D-Phe^7, D-Trp^9]-Substance P (DPDPDT) (Sigma-Aldrich Chemie Gmbh, Steinheim, Germany), in a dose of 5 μg/0.5 μl each were used. The drugs were dissolved in oxygen free distilled water or 2% water solution of vitamin C as an antioxidant (Krowicki and Hornby 2000), and the aliquots were stored at -70 °C until required.

Procedure

Surgery and EEG recordings were performed under deep urethane anesthesia (Urethane, Sigma-Aldrich, Chemie Gmbh, Steinheim, Germany; 1.2–1.6 g/kg, *i.p.*). The anesthesia level was controlled by counting the frequency of breathing. When necessary the anesthetic was added at the beginning of the recording session. With the rats mounted in a stereotaxic apparatus, bilateral hippocampal recording electrodes were located in the stratum moleculare of the upper blade of the dentate gyrus (stereotaxic coordinates depended on the size of an animal, AP: -3.6 to -3.8 mm, L: ± 2.0 to ± 2.1 mm, D: -3.0 to -3.7 mm relative to the bregma; according to atlas by Paxinos and Watson (1998)) and secured to the skull with stainless steel screws and dental acrylic. The electrodes were made of stainless steel wire of a 0.2 mm diameter, the entire length insulated except for the flat-cut tip. Stainless steel skull screws positioned over the olfactory bulbs, where electric activity is minimal, and fixed with dental acrylic served as ground and reference electrodes.

A 10-μl Hamilton microsyringe with a blunt tip needle of a 0.4 mm diameter (Aldrich, Milwaukee, WI, USA) was used for drug administration. The syringe was placed in the stereotaxic holder with a microinfusion pump (Kopf Instruments) above a hole drilled over the PPN (stereotaxic coordinates depended on the size of the animal, AP: -7.8 to -8.1 mm, L: ± 1.7 mm, D: -7.0 to -7.3 mm relative to the bregma; according to atlas by Paxinos and Watson (1998)).

The hippocampal EEG signal was recorded (sampling rate 240 Hz) using a Medicor electroencephalograph as a preamplifier (bandpass 0–70 Hz) with EEG DigiTrack software (Elmiko, Warsaw, Poland). The animals were maintained at a level of anesthesia at which spontaneous theta rhythm was not present in the hippocampal EEG, but could be elicited by a tail pinch produced with a plastic clamp (always the same). The clamp was positioned in the vicinity of the rat's tail base. Every tail pinch lasted 1 min. Before the cannula insertion three to four pinches were applied every 10 min to check for theta presence, and only animals with theta amplitude of at least 400 μV

in one or both hippocampi were included in the experiment.

After the completion of the control recording, the cannula was slowly lowered into the PPN and following a 10-min period of adjustment sensory stimulation was reapplied. Once the theta was elicited successfully, 0.5 μl of the vehicle was microinjected over 3 min. The tail pinch-induced theta elicited 10 min later served as control of the vehicle effect. Next, the cannula was gently removed, the vehicle in the microsyringe was replaced by SP (n=11) or DPDPDT (n=6) at a concentration of 5 μg /0.5 μl and the cannula was slowly lowered into the same place. Again, a 10-min period of adjustment was allowed following the insertion, after which the presence of the theta was checked with a tail pinch. Then, 0.5 μl of SP or DPDPDT (in separate animals) were injected into the PPN over 3 min and subsequently a tail pinch was reapplied every 10 min over 1 h. The EEG was recorded continuously throughout the experiment.

Data analysis

The spectral analysis of the hippocampal EEG in the theta (3.1–6 Hz) and delta (1–3 Hz) frequency bands was performed off-line with the discrete Fourier transform (DFT, resolution 0.01 Hz) calculated by Chirp-Z algorithm on 6–10 artifact-free 5-s epochs randomly chosen from 60-s records of the sensory stimulation. The following EEG records were analyzed: one preceding the cannula insertion into the PPN (preinjection baseline), 10 min postvehicle (control of a vehicle effect), and 10, 20, 30, 40, 50, and 60 min after drug administration.

Two power spectrum measures: the maximal peak power (μV^2, Pmax) and the frequency corresponding to the maximal peak power (Hz, Fmax) were assessed. To eliminate an inter-subject variability both Pmax and frequency were normalized: Pmax was expressed as a percentage of the preinjection baseline value taken as 100%, and the frequency was normalized to 4 Hz, because a preliminary analysis of the maximal amplitude in the theta band in all the rats revealed that the mean (± SD) corresponding frequency was 4 ± 0.07 Hz in the preinjection conditions and that its distribution was normal.

The effects of the intracerebral injections on Pmax and Fmax were statistically analyzed using one-way analysis of variance (ANOVA) with factor "experimental condition" (preinjection, 10 min postvehicle, 10, 20, 30, 40, 50 and 60 min postdrug) followed by the *post hoc* Tukey's test (at P<0.05). If a tested measure (Pmax or Fmax) differed significantly from both the preinjection and vehicle values it was considered as an effect of the drug. Following McNaughton and coauthors (1995) suggestion only differences of Fmax ≥ 0.4 Hz (mean) were considered as functionally meaningful. Comparisons of Pmax and Fmax for hippocampi ipsi- and contralateral to the injection were performed using the Independent-Samples Student's t-test. In cases when the differences in the quality (amplitude) of the control hippocampal theta in the two hemispheres were found, the effect of the intracerebral injection was analyzed only in unilateral hippocampus (ipsi- or contralateral), the one which fulfilled the criterion of the theta amplitude ≥ 400 μV.

Histology

At the end of each experiment, the animals were given an overdose of anesthetic. Then, electrolytic lesions (anodal current of 200 μA/20 s) were performed through the hippocampal electrodes to visualize their tips locations. Next, the rats were intracardially perfused with 0.9% saline (200 ml) followed by 10% formalin (200 ml). The brains were removed, fixed in 10% formalin, sectioned at 60 μm, and the placement of the hippocampal electrodes and microinjection cannulas tips in the PPN were confirmed histologically. Additionally, to visualize the spread of the fluid around the injection cannula 2% (in saline) alcian blue 8GX (Fluka, Switzerland) in a volume of 0.5 μl was injected into the PPN and the diffusion area was measured under a light microscope (Nikon Eclipse E600) in a sample rat.

RESULTS

Histological verification

Out of 17 animals tested 15 had proper localization of the cannula tips in the PPN. In 2 rats (one injected with SP and one with DPDPDT) the cannulas were misplaced and localized above the PPN in the retrorubral field or the cuneiform nucleus (Fig. 1). These animals were used as control for anatomical specificity of the effects observed and were excluded from statistical analysis.

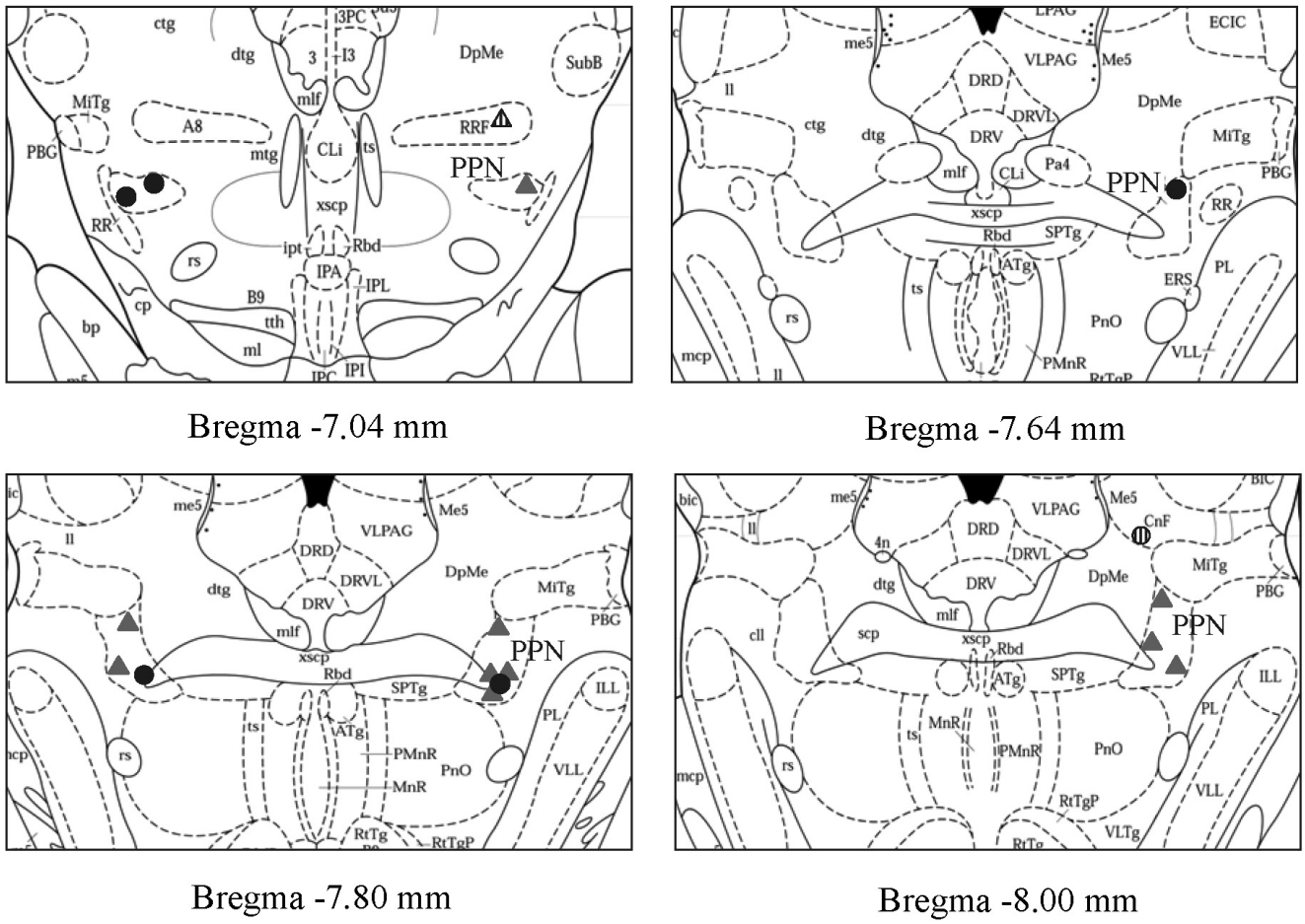

Fig. 1. Localization of SP and DPDPDT microinjections sites superimposed on plates taken from the atlas by Paxinos and Watson (1998). Explanations: solid triangles – SP cannulas within the PPN; dashed triangle – SP cannula outside the PPN; solid circles – DPDPDT cannulas within the PPN; dashed circle – DPDPDT cannula outside the PPN.

The hippocampal electrodes (not shown) from which the theta rhythm was of an amplitude ≥ 400 μV were localized in the stratum moleculare of the upper blade of the dentate gyrus (either bilaterally symmetrical or unilaterally). In the cases when the theta amplitude was ≤ 400 μV the electrodes were found in the upper zone of the stratum oriens of CA1 or slightly above it, in the stratum radiatum of the hippocampal fissure and in the hilus of the dentate gyrus. The latter were excluded from the analysis.

As found in a sample rat injected with the alcian blue dye in the PPN, the spread of the injection was approximately 0.6/0.4/0.8 mm along the AP/L/D axes.

Substance P

Ten animals with proper localization of the intra-PPN cannula were analyzed. Bilateral hippocampi were analyzed in 4 rats, only ipsilateral ones in 2 rats and only contralateral ones in 4 rats.

The effects of unilateral microinjection of 5 μg of SP into the PPN on the sensory-induced hippocampal theta rhythm were evident in both hippocampi even upon visual inspection of the EEG records as shown for an example animal in Fig. 2. SP caused suppression of the theta, which was always accompanied by an increase in the delta activity. In the power spectra there was a loss of a definite peak at theta frequency range with wide distribution of power across the tested frequencies (Fig. 2).

Figure 3 presents changes in *P*max and the corresponding *F*max after SP administration in the theta and delta bands. SP significantly decreased theta *P*max in the hippocampus ipsi- ($F_{7,358}$=17.69, P<0.001) and contralateral ($F_{7,496}$=29.10, P<0.001) to the intra-PPN injection. A marked decline of theta *P*max to 53.0 ± 5.3% and 46.3 ± 5.6% of the preinjection value in the

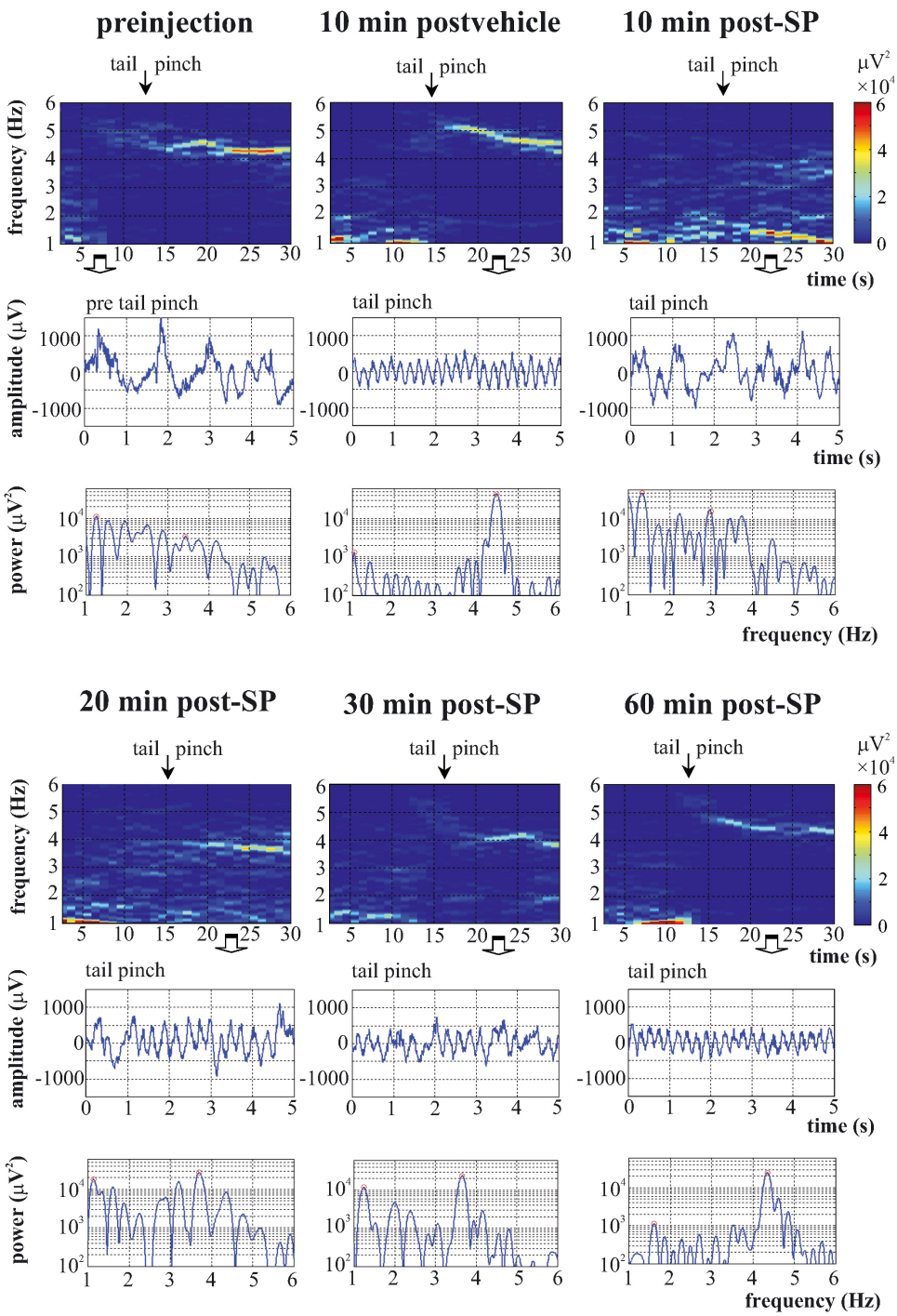

Fig. 2. The course of the experiment in a typical rat receiving intra-PPN injection of SP (hippocampus ipsilateral to the injection). Upper panels: spectrograms (window width: 5 s, step: 2 s) depicting transitions from the spontaneous to tail pinch-induced hippocampal EEG activity in the preinjection conditions, 10 min postvehicle, and at different time points post-SP. Note a stripe which appears in the theta frequency range (3.1–6 Hz) following tail pinch in the preinjection and postvehicle conditions, and does not in the post-SP conditions. Central panels: a record of the spontaneous hippocampal EEG in the preinjection conditions (pre tail pinch) and records of a tail pinch-evoked hippocampal EEG activity in the postvehicle and post-SP conditions (tail pinch) (⊓ indicates the fragments of EEG spectrograms corresponding to the records in the central panels). Note a theta loss 10–30 min post-SP. Lower panels: power spectra relevant to the EEG samples in the central panels. Note a decrease in theta peak power post-SP and an increase in delta peak power (frequency range 1–3 Hz).

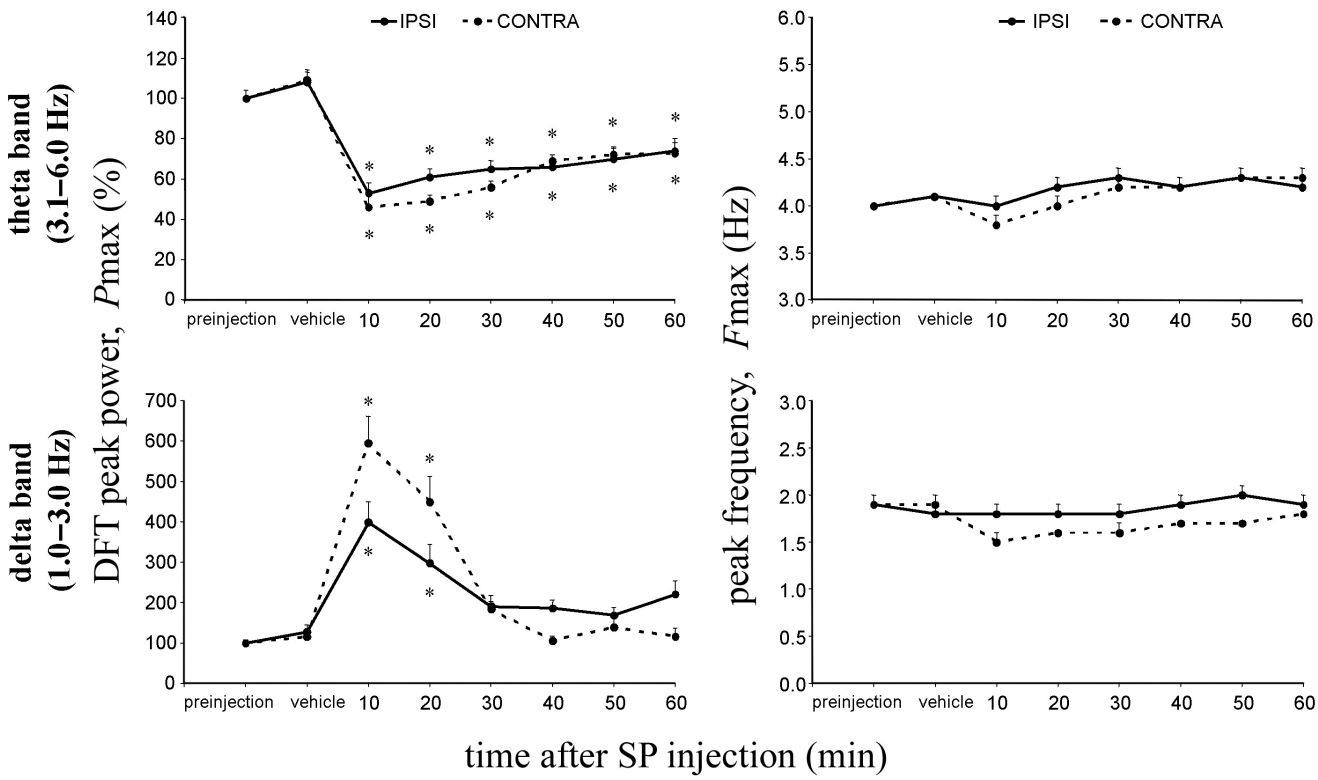

Fig. 3. Effect of unilateral injection of SP (5 μg/0.5 μl) to the PPN on *P*max (left graphs) and the corresponding *F*max (right graphs) of the tail pinch-evoked EEG signal in the hippocampus ipsi- (*n*=6) and contralateral (*n*=8) to the injection side. Data is presented as means ± SE. (*) $P<0.05$ as compared to both the preinjection and vehicle levels. Note a decrease in theta *P*max and an increase in delta *P*max starting 10 min after SP administration.

hipocampus ipsi- and contralateral, respectively, was seen as early as 10 min after drug administration. Although theta *P*max was slowly returning to the preinjection level and reached 74.0 ± 5.5% (ipsi-) and 72.9 ± 5.4% (contralaterally) of the baseline it was still significantly reduced 60 min post-SP.

The diminution of theta *P*max was accompanied by a marked increase in delta *P*max both ipsi- ($F_{7,365}$=9.85, $P<0.001$) and contralaterally ($F_{7,505}$=8.05, $P<0.001$), and reached, respectively, 398.9 ± 50.5% and 594.7 ± 67.2% of the baseline 10 min postinjection. The significant increase in delta *P*max persisted over 20 min (Fig. 3).

SP effect on *F*max was not observed in the theta nor in the delta band (Fig. 3).

SP tended to affect the theta and delta activities in the contralateral hippocampus more profoundly than in the ipsilateral one. Inter-hemispheric comparisons revealed significantly lower theta *P*max on the contra- than ipsilateral side 20 min following SP (respectively 48.5 ± 3.3% and 60.7 ± 4.4%, $P<0.05$, Student's *t*-test). Delta *P*max was significantly higher contra- than ipsilaterally 10 min postinjection (respectivelly 594.7 ± 67.2% and 399.0 ± 50.5% of the baseline, $P<0.05$, *t*-Student's test) (Fig. 3).

No effect of vehicle administration was ever observed. Neither was a change of the theta found after SP injection into the retrorubral field.

DPDPDT

Five animals with proper localization of the intra-PPN cannula were analyzed. Bilateral hippocampi were analyzed in all 5 rats.

Intra-PPN administration of 5 μg of DPDPDT totally abolished the tail pinch-elicited theta activity in both hippocampi in all the animals. Figure 4 shows the course of the experimental session in an example rat. A shift of the peak power from the theta to delta frequency range is clearly visible. The hippocampal EEG became dominated by delta activity. The effect of

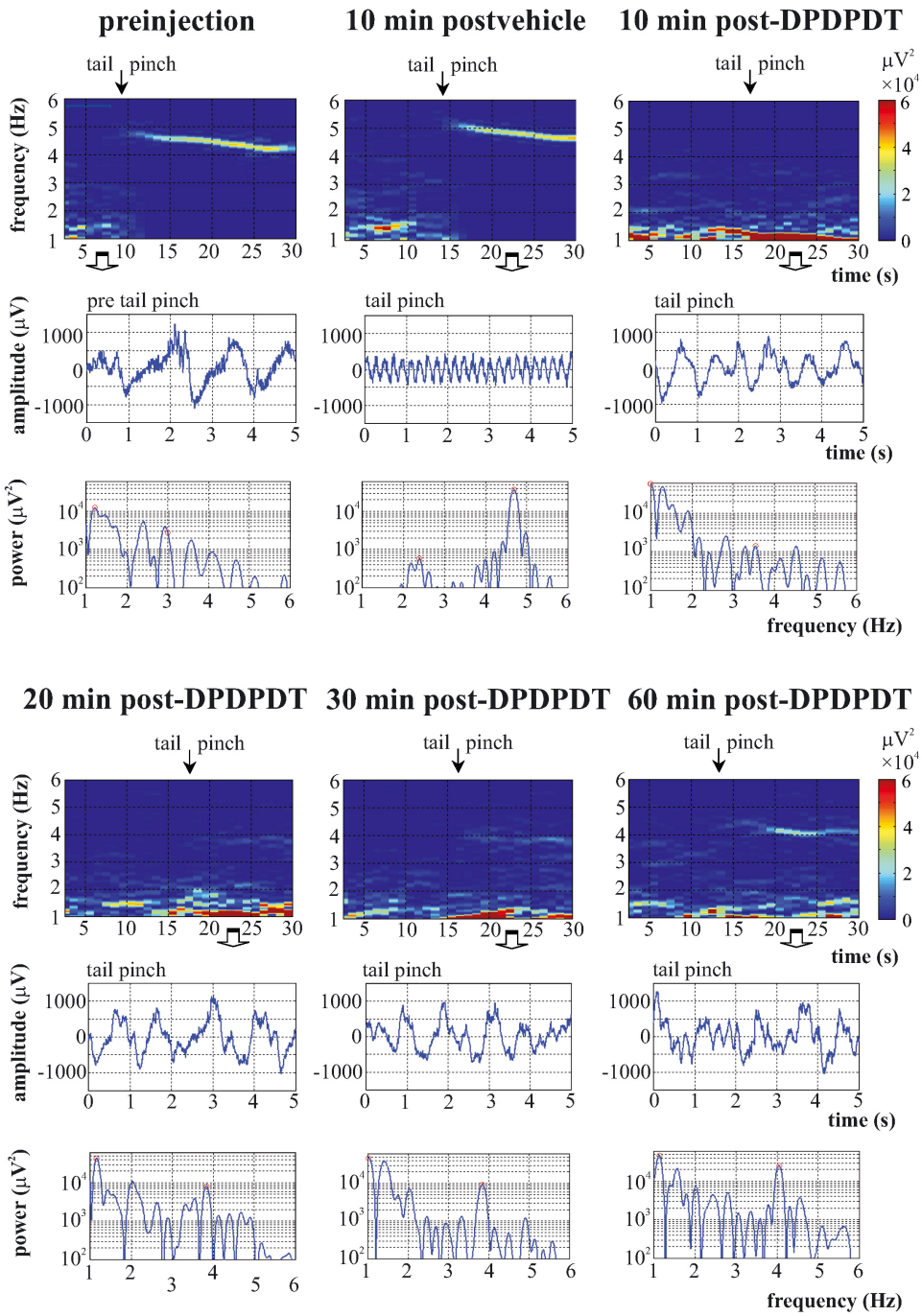

Fig. 4. The course of the experiment in a typical rat receiving intra-PPN injection of DPDPDT (hippocampus ipsilateral to the injection). Upper panels: spectrograms (window width: 5 s, step: 2 s) depicting transitions from the spontaneous to a tail pinch-induced hippocampal EEG activity in the preinjection conditions, 10 min postvehicle, and at different time points post-DPDPDT. Note a stripe which appears in the theta frequency range (3.1–6 Hz) following tail pinch in the preinjection and postvehicle conditions, and does not in the post-DPDPDT conditions. Central panels: a record of the spontaneous hippocampal EEG in the preinjection conditions (pre tail pinch) and records of a tail pinch-evoked hippocampal EEG activity in the postvehicle and post-DPDPDT conditions (tail pinch) (⊓ indicates the fragments of EEG spectrograms corresponding to the records in the central panels). Note a theta loss post-DPDPDT. Lower panels: power spectra relevant to the EEG samples in the central panels. Note a decrease in theta peak power post-DPDPDT and an increase in delta peak power (frequency range 1–3 Hz).

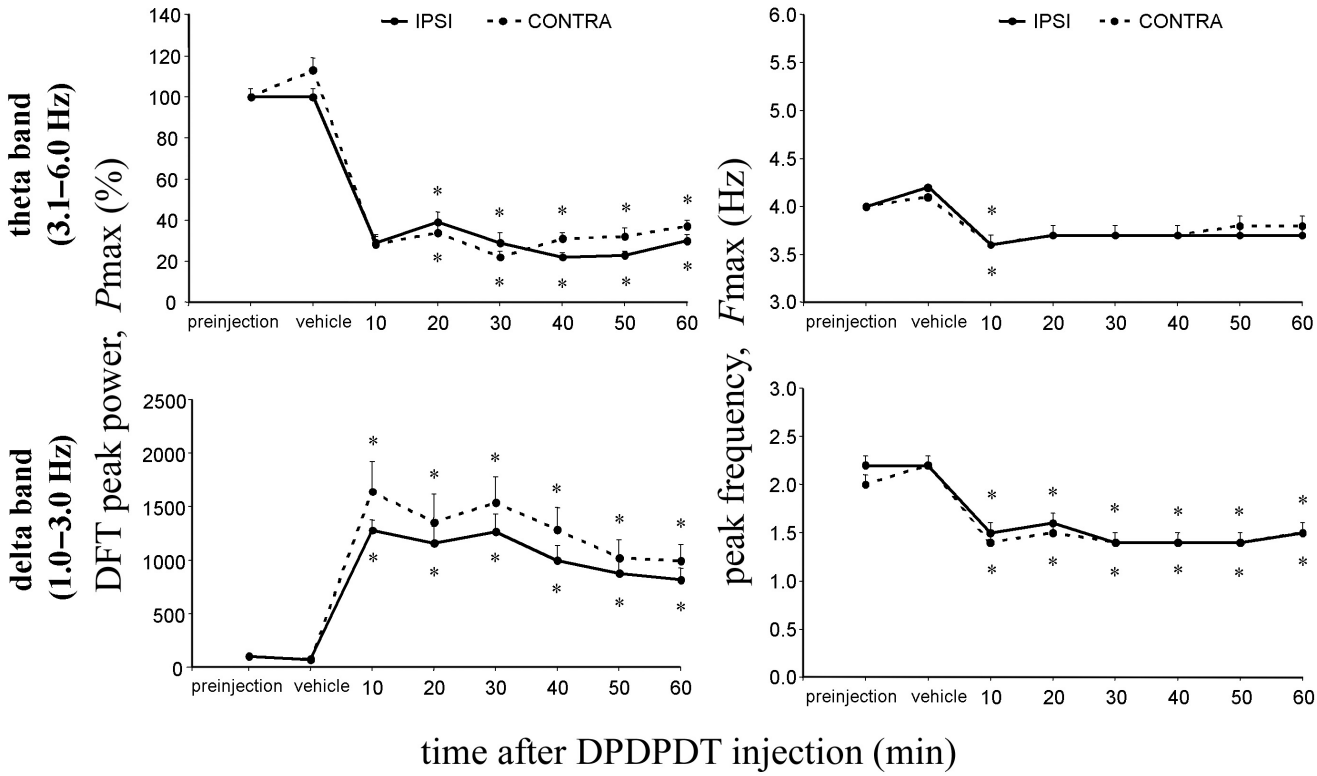

Fig. 5. Effect of unilateral injection of DPDPDT (5 μg/0.5 μl) to the PPN on *P*max (left graphs) and the corresponding *F*max (right graphs) of the tail pinch-evoked EEG signal in the hippocampus ipsi- and contralateral to the injection side (n=5). Data is presented as means ± SE. (*) P<0.05 as compared to both the preinjection and vehicle levels. Note a decrease in theta *P*max, an increase in delta *P*max, and a decline of *F*max starting 10 min after DPDPDT injection.

DPDPDT was longlasting and persisted until the end of the 60-min experimental session.

Figure 5 shows changes in *P*max and *F*max in the theta and delta bands following DPDPDT administration. There was a substantial attenuation of theta *P*max in the hippocampus ipsi- ($F_{7,355}$=68.62, P<0.001) and contralateral ($F_{7,356}$=70.76, P<0.001) to the intra-PPN injection. Theta *P*max lowered significantly to 29.0 ± 4.0% and 28.1 ± 4.1% of the preinjection value on the ipsi- and contralateral side, respectively, 10 min after SP administration and continued at this level for 60 min. Furthermore, DPDPDT led to a significant reduction of theta *F*max both ipsi- ($F_{7,355}$=9.81, P<0.001) and contralaterally ($F_{7,356}$=8.29, P<0.001). Theta *F*max fell by about 0.4 Hz as compared to the preinjection baseline reaching 3.6 ± 0.1 Hz 10 min following DPDPDT in both the hippocampi.

The suppression of the theta activity was accompanied by a marked increase in delta *P*max in the hippocampus ipsi- ($F_{7,335}$=15.25, P<0.001) and contralateral ($F_{7,336}$=8.46, P<0.001) to the intra-PPN injection (Fig. 5). Delta *P*max rose to 1279.7 ± 93.8% and 1638.8 ± 284.7%, respectively, as soon as 10 min post-DPDPDT. It started to decline 50 min postinjection, yet still above the preinjection level until the end of the experiment, when it reached 818.1 ± 105.5% (ipsi-) and 993.9 ± 156.1% (contralaterally) 60 min postinjection. DPDPDT caused significant falls of delta *F*max in the hippocampus ipsi- ($F_{7,335}$=21.54, P<0.001) and contralateral to the injection ($F_{7,336}$=19.55, P<0.001). Delta *F*max dropped from 2.2 ± 0.1 Hz in the preinjection conditions to 1.5 ± 0.1 Hz 10 min following DPDPDT on the ipsi- and from 2.0 ± 0.1 Hz to 1.4 ± 0.1 Hz on the contralateral side, respectively (Fig. 5). It remained significantly lowered over 60 min postinjection: by 0.8–0.7 Hz ipsi- and by 0.5–0.6 Hz contralaterally.

There were no significant differences between the hippocampus ipsi- and contralateral to the injection after DPDPDT, except for the theta band 40 and

50 min postinjection, when DPDPDT resulted in a deeper decline of theta *P*max ipsi- than contralaterally: 21.7 ± 2.4% vs. 31.2 ± 3.3% after 40 min and 22.9 ± 2.3% vs. 32 ± 3.5% after 50 min ($P<0.05$) (Fig. 5).

No effect of vehicle administration was found in this group. Neither did an injection of DPDPDT into the cuneiform nucleus change the theta rhythm.

DISCUSSION

The principal finding of this study is that unilateral intra-PPN administration of two substances acting on tachykinin receptors, namely SP and its analogue DPDPDT, bilaterally suppressed theta and enhanced delta activity in the hippocampus of urethane anesthetized rats. DPDPDT exerted deeper and longer effects than SP, and influenced both frequency and power, while SP affected predominantly power. Theta inhibition was manifested as its peak power decline and, following DPDPDT, also as the drop of the corresponding peak frequency. Delta augmentation was expressed as an increment of its peak power, which, after DPDPDT, was also accompanied by the slowdown of delta waves. These results indicate that the tachykinin system in the PPN is significantly involved in the generation of the hippocampal theta rhythm.

In previous experiments by Kosinski and coauthors (1981, 1984) SP was found to promote the theta. Methodological differences between their and our experiments can account for these discrepancies. Firstly, Kosinski and coauthors (1981, 1984) used a C-terminal fragment of SP, hexapeptide SP_{6-11}, which may differ in its biological activity from SP used in this study. There is experimental evidence indicating differences in the biological activity between SP and SP_{6-11} fragment. For example, SP shows higher affinity for NK1 receptors than SP_{6-11} (Regoli et al. 1989, Saria 1999), SP_{6-11} causes lower desensitization of rat NK1 receptors than SP (Vigna 2001) and does not block amnestic effects of diazepam as SP does in rats (Costa and Tomaz 1998). Secondly, tachykinins' effect may be dose-dependent. The potency of SP influence on the local brain electrical activity was suggested to be proportional to its content in the relevant brain region (Ogata 1979). Although, it is difficult to compare *i.c.v.* 50 µl (500 nmol/ml) of SP_{6-11} in Kosinski's and coauthors' (1981, 1984) work with intra-PPN 5 µg of SP in our study, a possibility that the effective dose in the relevant brain circuitry was different to the point of exerting opposite influences cannot be excluded. Next, the drugs acted at different levels of the theta synchronizing system in both studies. Kosinski and coauthors (1981, 1984) believed that intraventricular SP_{6-11} operated mainly at the septal level. In our study, SP acted in the PPN, which provides cholinergic (Hallanger and Wainer 1988) and SP (Szeidemann et al. 1995) innervation to the lateral septum but is generally thought to operate through the rostral pontine nucleus theta generator (e.g., Vertes et al. 1993).

SP is colocalized with ACh in the cholinergic PPN neurons (Kohlmeier et al. 2002, Standaert et al. 1986, Szeidemann et al. 1995), which are regarded as main efferents from the PPN to the other theta-relevant structures (Hallanger and Wainer 1988, Rodrigo-Angulo et al. 2005, Semba et al. 1990, Vertes et al 1993). It is also present in the PPN noncholinergic (Kohlmeier et al. 2002), and presumably GABA- and glutamatergic neurons. Considering the fact that activation of PPN cholinergic transmission evokes the theta (Kinney et al. 1998, Vertes et al 1993), the opposite effect i.e., its inhibition, could account for theta suppression. SP is known to mediate increases of GABA release *via* NK1 receptors in different brain regions e.g., hippocampal CA1 region (Kouznetsova and Nistri 1998, Ogier and Raggenbass 2003) and the rat nucleus tractus solitarius (Bailey et al. 2004). Thus, SP could inhibit PPN ACh neurons by increasing the excitability of its GABA-ergic inputs. Indeed, $GABA_A$ agonist, muscimol, was found to suppress the theta when administered directly into the PPN (Nowacka and Trojniar 2000).

Moreover, in hippocampal dentate gyrus granule cells, SP produces a robust enhancement of NMDA channel function, prolonging the opening of the channels, and thus provoking an enhancement of the glutamate release (Lieberman and Mody 1998). Yet, in the CA1 pyramidal cells region SP agonist depressed glutamatergic events (Kouznetsova and Nistri 1998). Electrical activity of cellular components in the hippocampus is controlled by a complex network involving GABA, glutamate and SP modulation (Kouznetsova and Nistri 1998). In the case of the PPN, activation of its glutamatergic as well as GABA-ergic transmission inhibits the theta (Nowacka and Trojniar 2000). We hypothesize that the efferent PPN neurons are regulated by the internal PPN circuitry with GABA interneurons inhibiting cholinergic effer-

ents, and glutamate exciting the GABA interneurons. SP may modulate activity of each component of this circuitry but the theta loss suggests prevailing excitation of the theta inhibitory component (GABA- and glutamatergic).

The SP analogue, DPDPDT, evoked similar but longer and deeper theta suppression than that caused by SP. In fact, DPDPDT has been reported to act as a SP antagonist, a partial agonist, or even a full agonist. For example: DPDPDT microinjected into the prefrontal cortex in rats attenuated carbachol-elicited "boxing" behavior, that was potentiated by SP (Stivers and Crawley 1988); SP microinjected into the locus coeruleus, periaqueductal gray matter and parabranchial nucleus induced a pressor response, which was reduced by preinjection of DPDPDT (Wang et al. 1997); the pressor response to glutamate induced by SP mincroinjection was also reduced by administration of DPDPDT into nuclei, which contain SP-immunoreactive cell bodies, nerve terminals and receptors involved in stress and behavior regulation, such as the paraventricular, ventromedial and dorsomedial hypothalamic nuclei (Wu et al. 1999). A suggestion that DPDPDT was acting as a partial agonist relative to SP was made by Bailey and Jordan (1984), when they found that DPDPDT similarly to SP contracted the rat colon preparation, and that the maximal response exhibited by this analogue was about 30% that of the SP. Agonistic responses to DPDPDT were observed in the rat superior cervical ganglion and the rabbit external jugular vein, and were considered to be mediated directly through the same receptor system as SP (Hawcock et al. 1982).

The discrepancies between DPDPDT effects on different tissues in rats may result from a diversity of multiple tachykinin receptors on target cells. Three subtypes of tachykinin receptor NK1, NK2 and NK3 have been identified so far, and a variant form of the NK3, referred to as NK3B or NK4. The former three (Saffroy et al. 2003) and the NK4 mRNA (Donaldson et al. 2001) were detected in the adult rat brain. While SP acts on NK1–NK3 receptors, with the highest affinity for NK1 (NK1>NK2>NK3), analogs of SP can be simultaneously agonist at one biding site and antagonist at the other biding site associated with the NK1 receptor (Sachon et al. 2002). This data suggests that SP and DPDPDT may differ in their affinity to different tachykinin receptor subtypes, whose distribution varies among the tissues, and whose activation may lead to different effects. Moreover, endogennous SP co-released with other neurotransmitters may well exert its modulatory effects *via* the feedback mechanisms, whose outcome may vary in the case of the presence of exogenous SP or DPDPDT. This may possibly explain the stronger inhibitory effect of DPDPDT observed in the present study, which concerned both theta amplitude and frequency.

To summarize, in the present experiment both substances acting on the tachykinin receptor systems in the PPN caused suppression of the theta. The circuitry through which SP exerts its effect in the PPN can be only hypothetical at present. The fact that SP was found in both cholinergic and noncholinergic PPN neurons (Kohlmeier et al. 2002, Standaert et al. 1986, Szeidemann et al. 1995) makes a supposition on the exact operation of this circuitry even more vague. As SP is a depolarizing modulator (Ito et al. 2002), a most probable hypothesis is that it suppresses the theta through activation of the theta inhibiting (Nowacka and Trojniar 2000) GABA interneurons in the PPN (Ford et al. 1995) as it does in the hippocampal formation and other structures (e.g., Dreifuss and Raggenbass 1986, Kouznetsova and Nistri 1998). SP may also enhance NMDA-related glutamatergic transmission, which was found to mediate the theta-inhibiting influences in the PPN (Nowacka and Trojniar 2000).

It was found in the *in vitro* study (Hara and Harris 2002) that urethane anesthesia, used in the present as well as in the majority of other studies on the brainstem regulation of the theta, affects recombinant $GABA_A$, NMDA, and neuronal nicotinic cholinergic receptors expressed in Xenopus oocytes. This should be taken into consideration when interpreting the results of the intracerabral microinjection experiments performed under urethane. Therefore, it would be worthwhile verifying at least some of the intra-PPN neurotransmitter microinjection studies on conscious, freely behaving animals.

CONCLUSION

The tachykinin system in the PPN is significantly involved in the regulation of the hippocampal type 2 theta rhythm, possibly through depolarization of the PPN inhibitory interneurons, which control activity of the PPN output neurons.

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