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Influence of CCK-8 and yohimbine on supraspinal modulation of nociceptive process

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Abstract. We studied the effect of cholecystokinin (CCK-8) and yohimbine - α-2-adrenoreceptor antagonist administered locally into lateral reticular formation (LRF) on the bioelectrical response to the pain stimulation. The experiments were carried out on 8 concious rabbits with bilaterally implanted electrodes into: motor-sensory cortex, ventro-postero-lateral thalamic nuclei, hippocampus and LRF. Nociceptive stimulation was performed by means of electrical pulses applied to the front paw. Bioelectrical activity (BA) of the chosen brain structures was analysed by means of spectral analysis (FFT) and directed transfer function (DTF). The results of our study may suggest that supraspinal administration of CCK-8 together with yohimbine have inhibitory effect on nociceptive transmission.

Short communication

Key words: CCK-8, yohimbine, nociception, lateral reticular formation, FFT, DTF

Noradrenaline and CCK-8 are known as neurotransmitters involved in pain modulation in CNS (Faris et al. 1983, Sagen and Proudfit 1985, Kuraishi et al. 1987, Dourish et al. 1990). High concentrations of α-2-adrenergic and CCK receptors are localised in many brain regions closely connected with pain transmission i.e. cerebral cortex, hypothalamus, hippocampus, amygdala, brainstem and dorsal horn of spinal cord (Pertovaara 1993, Crawley and Corwin 1994). Recently, much experimental work has drawn attention to the role of α-2-adrenoreceptors of the medullary lateral reticular formation (LRF) in antinociception (Murphy and Behbehani 1993, Mansikka and Pertovaara 1995). Brainstem LRF is a structure with noradrenaline-containing spinally projecting neurones (Hall et al. 1982). There are some findings suggesting that LRF is involved in tonic descending inhibition of spinal nociception and it is also a relay for antinociceptive signals descending from the periaqueductal gray (PAG) to the spinal cord (Hall et al. 1982, Sandkuhler and Gebhart 1984). In turn, PAG has high concentration of CCK receptors. CCK is an important excitatory neurotransmitter in this brainstem region (Rosén et al. 1992). There are many conflicting data referring to the effects of α-2-adrenergic agents applied supraspinally on the nociceptive process. Both α-2-adrenergic receptors agonists (e.g. clonidine, flupiritine, medetomidine) and antagonists (e.g. yohimbine, idazoxan) were observed to have opposed actions (inhibition or facilitation) on the pain transmission depedently on analysed structure (Sagen and Prodfit 1985, Izenwasser and Kornetsky 1990, Murphy and Behbehani 1993, Pertovaara 1993). While the involvement of spinal opiod system in α-2-induced analgesia was suggested in some data (Mastrianni et al. 1989, Izenwasser and Kornetsky 1990), many studies indicate that opioids and α-2-adrenoreceptors produce analgesia through independent receptors system (Spanos et al. 1989, Xu and Wiesenfeld-Hallin 1993). On the other hand, CCK is commonly accepted to be endogenous antagonist of opioid-induced analgesia at the level of spinal cord (Faris et al. 1989, ONeill et al. 1990, Tseng and Collins 1992, Noble et al. 1993). Our previous investigations showed that both supraspinal activation and blocade (to a lesser extent) of α-2-adrenergic receptors had an inhibitory effect on nociceptive transmission. Similarly, CCK-8 administered into LRF had a dose dependent inhibitory effect on nociceptive process. In turn, clonidine microinjected together with CCK-8 into LRF had opposed effect and enhanced the pain transmission (unpublished data). The aim of the present study was to evaluate the influence of administration of yohimbine together with CCK-8 into LRF on nociceptive process.

The experiments were carried out on 8 male rabbits weighing 3.0-3.5 kg. Animals were anaesthetized with morphine sulphate 10-15 mg/kg and chlorpromasine 25-40 mg/kg. The stainless steel electrodes (0.3 mm in diameter, connected in pairs, distance between tips was 0.3 mm) were implanted bilaterally into: the motor-sensory cortex (MSC), ventro-postero-lateral thalamic nuclei (NVPL), hippocampus (HIP), and LRF where additionally a cannula was implanted. Three weeks after the surgery (the recovery and habituation period), we started the nociceptive stimulation (NS) on restrained but concious animals located in a sound-proof chamber. NS was performed by means of electrical pulses (60 Hz, 8 mA, 5 s) applied to the front paw using intracutaneous electrodes. Bioelectrical activity (BA) of the analysed brain structures was registered before and after NS in control animals and after administration of drugs. The drugs: yohimbine (3 μl, conc. 7 mg/ml) together with CCK-8 (in the doses: 100 and 200 ng per rabbit) were applied locally into LRF 5 min before NS. The 10 second EEG recordings before after NS were analysed by means of the spectral analysis (Fast Fourier Transform - FFT) to determine power spectra for frequency bands (slow waves, delta, theta, alpha and beta) and by autoregressive method (AR) for multichannel model. Parameters achieved in AR made it possible to calculate the directed transfer functions (DTFs). DTFs were counted in the frequency range 0-50 Hz for all channels. DTF gives information on the frequency content and direction of BA flows between the studied brain structures

(Kamiński and Blinowska 1991, Kamiński et al. 1995). DTF value at a given frequency is meant to describe the percent of the signal of this frequency recorded from one electrode, as appearing with some delay in the recording registered by a second electrode at the target point (Bekisz and Wróbel 1993).

The results of DTF analysis are presented in the form of schemata demonstrating the layout of BA flows between the analysed structures. Significant flows are shown by means of arrows. Typical outputs of DTF analysis (in the form of frequency dependent plots) are placed together with an adequate schema. These schemata are additionally supplied with the comparisons showing statistically significant differences in BA flow between structures.

The layout of BA flows before NS is presented in Fig.1.

The changes of BA induced by NS are shown in Fig. 2.

Figure 3 demonstrates differences between the effect of administration of yohimbine and CCK-8 dependently on the dose of CCK-8 (100 ng and 200 ng) on BA of the analysed structures.

The FFT analysis showed that the drugs administration (CCK-8 in both doses) caused the following changes of BA in MSC: increase of the power spectra for slow and delta waves and decrease - for alpha waves in comparison with BA before the drugs administration (Fig. 1D and E). Similar changes of BA in cortex were observed after NS in

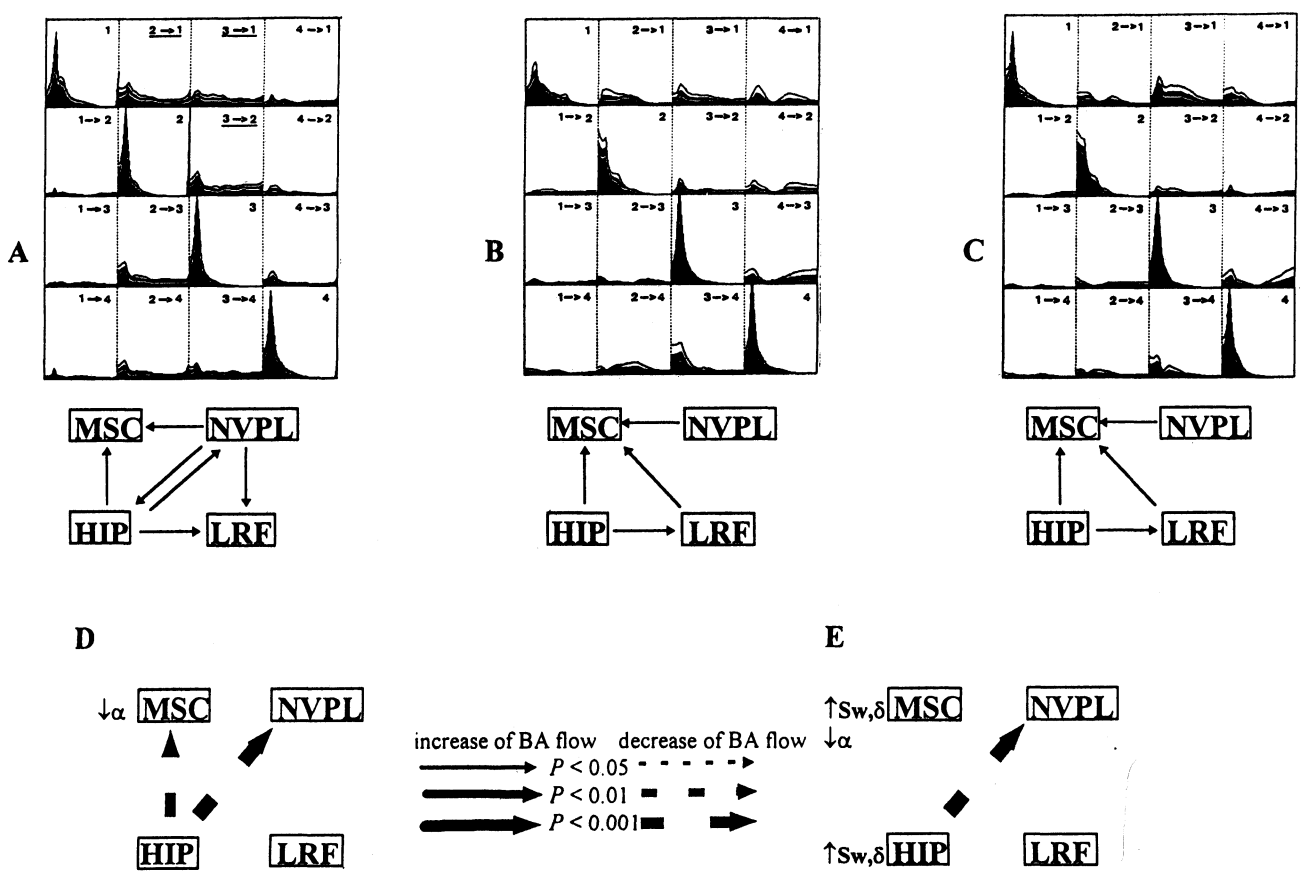

Fig. 1. The layout of BA flow between the studied brain structures before NS: A, BA before the drugs administration; B, BA after administration of yohimbine together with CCK-8 in the 100 ng dose; C, BA after administration of yohimbine together with CCK-8 in the 200 ng dose; D, statistically significant differences between A and B; E, statistically significant differences between A and C. In A, B and C the typical output of DTF (above) and the schema of BA flow (below). The direction of the significant BA flow is shown by arrows. ↑ increase of the power spectra value for the analysed waves, ↓ decrease of the power spectra value for the analysed waves. 1-MSC, motor-sensory cortex; 2-NVPL, ventro-postero-lateral thalamic nuclei; 3-HIP, hippocampus; 4-LRF, lateral reticular formation.

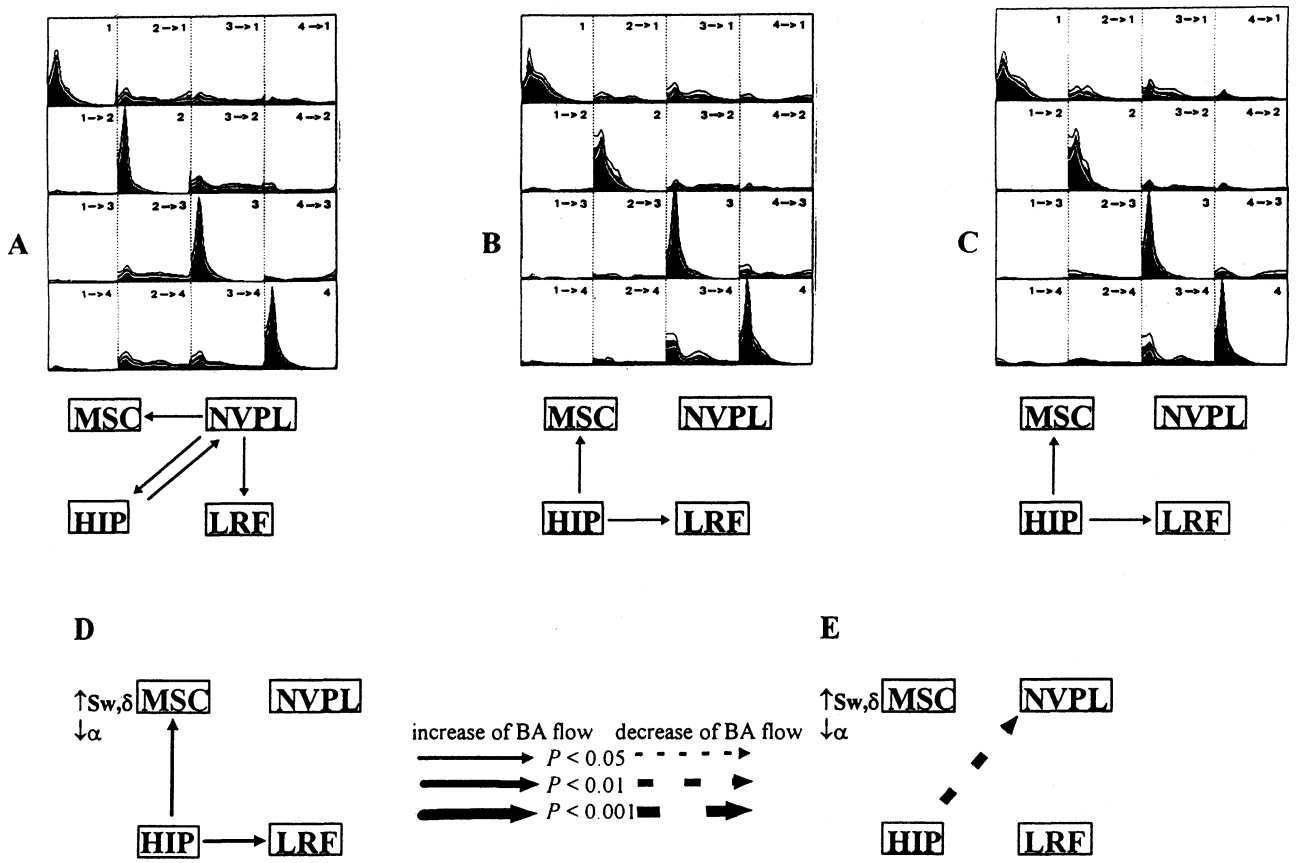

Fig. 2. The layout of BA flow between the studied brain structures after NS: A, BA before the drugs administration; B, after administration of yohaimbine together with CCK-8 in the 100 ng dose; C, BA after administration of yohimbine together with CCK-8 in the 200 ng dose; D, statistically significant differences between A and B; E, statistically significant differences between A and C. In A, B and C the typical output of DTF (above) and the schema of BA flow (below). The direction of the significant BA flow is shown by arrows. ↑ increase of the power spectra value for the analysed waves, ↓ decrease of the power spectra value for the analysed waves. 1-MSC, motor-sensory cortex; 2-NVPL, ventro-postero-lateral thalamic nuclei; 3-HIP, hippocampus; 4-LRF, lateral reticular formation.

the case of the microinjection of yohimbine together with CCK-8 in the 100ng dose (Fig. 3B). In contrary, NS before the drugs administration increased BA of the analysed structures (Fig. 3A). These findings may suggest that yohimbine and CCK-8 (100 ng dose) had an inhibitory effect on the bioelectrical response of the studied brain regions after NS. Additionally, the DTF analysis reveales that after NS these drugs reduced the BA flows: from HIP to MSC and NVPL (in the 100 ng dose of CCK-8) and from HIP to NVPL (in the 200 ng dose of CCK-8) in comparison with BA after NS before the drugs injection (Fig. 2D and E). From these results, it appears that BA of HIP was suppressed. HIP has many neuronal connections with noradrenergic regions of the brainstem. Noradrenaline released from nerve endings inhibits spontaneous activity of hippocampal neurones (Storm-Mathisen and Ottersen 1984), thus yohimbine may enhance the release of noradrenaline by blockade of α-2-adrenergic receptors in HIP.

We can see that NS induced similar layout of the BA flows before and after drugs administration, i.e. the characteristic increase of the flow from NVPL and HIP to MSC in comparison with spontaneous BA in DTF analysis (Fig. 3A, B and C). However, the statistical significancy of that increase is lower after drugs administration. We also observed the enhancement of BA flow: from LRF to NVPL (CCK-8 in 100 ng dose) and from LRF to MSC (CCK-8 in

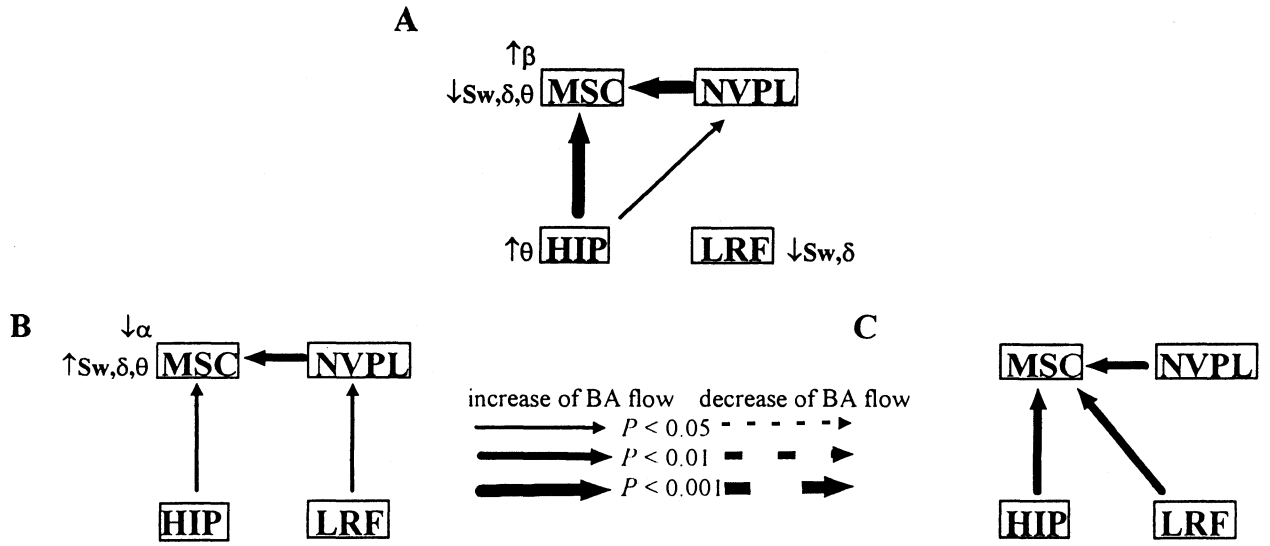

Fig. 3. Statistically significant differences between the layouts of BA flow before and after NS: A, before the drugs administration; B, after administration of yohimbine together with CCK-8 in the 100 ng dose; C, after administration of yohimbine together with CCK-8 in the 200 ng. ↑ increase of the power spectra value for the analysed waves, ↓ decrease of the power spectra value for analysed waves. MSC, motor-sensory cortex; NVPL, ventro-postero-lateral thalamic nuclei; HIP, hippocampus; LRF, lateral reticular formation.

200 ng dose) (Fig. 3B and C). These data demonstrate that the microinjection of studied drugs increased activity of LRF after NS. It is in agreement with some works reported the complex role of LRF in modulation of nociceptive transmission (Hall et al. 1982, Sandkühler and Gebhart 1984, Murphy and Behbehani 1993, Mansikka and Pertovaara 1995).

In conclusion, our findings may suggest that yohimbine administered together with CCK-8 (in both doses) into LRF have inhibitory effect on nociceptive process. The mechanism of this effect remains to be established.

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