Short communication

Effects of electro-acupuncture and physical exercise on regional concentrations of neuropeptides in rat brain

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Abstract

The effects of single or repeated treatments with manual acupuncture (ACU), electro-acupuncture (ELACU) or physical exercise on neuropeptide Y (NPY), neurokinin A (NKA), substance P (SP), galanin (GAL) and vasointestinal peptide (VIP)-like immunoreactivity (-LI) in different regions of the rat brain were studied. Initially the effect of microwave irradiation (MWI) was compared to decapitation on the recovery of neuropeptides, and significantly higher concentrations of SP-LI, NKA-LI and NPY-LI were found in the hippocampus, occipital cortex, pituitary and striatum following MWI. Repeated ELACU treatments significantly increased SP-LI, NKA-LI and NPY-LI in the hippocampus and NPY-LI in the occipital cortex. No changes were found in animals receiving ACU or performing physical exercise.

Keywords: Neuropeptide; Neuropeptide Y; Rat brain; Hippocampus; Electroacupuncture; Microwave irradiation

Although acupuncture has been used for the treatment of various diseases and pain alleviation for centuries in China, and primarily for painful conditions over a few decades in Western countries, its use has been based more on empirical observations than on scientific knowledge. Recent evidence, however, indicates that acupuncture (ACU) and electro-acupuncture (ELACU) as well as physical exercise may reduce pain and influence autonomic reactions via common afferent pathways [2]. These techniques of afferent stimulation activate numerous mediators in the central nervous system [7].

It has been proposed that endogenous opioids play an important role in mediating the effects of acupuncture in pain [12,23]. Administration of the opioid antagonist naloxone inhibited anti-nociception induced by single ELACU in mice [24] and antagonized the pain threshold increasing effect of acupuncture in humans [20]. Furthermore, single ELACU increased the levels of endorphins both in human [21] and in rat CSF [22]. Measurement of enkephalin in rat and rabbit brain showed significantly raised levels in the hypothalamus, striatum and CSF following 30 min of ELACU [34].

Controversial data strongly suggest that other mechanisms besides endogenous opioids also are responsible for the effects of acupuncture in pain. Chapman et al. reported an increase in pain threshold in humans following a single electro-acupuncture treatment which was unaffected by naloxone [6]. These results are supported by others showing a lack of reversal in both experimental and clinical pain [19]. Changes in other neurotransmitters such as 5-hydroxy tryptamine, catecholamines, acetylcholine and various hormones have been implicated in acupuncture induced anti-nociception [7,12]. An interesting group of neurotransmitters, with a possible role in pain and acupuncture, are the neuropeptides [13,17]. Still very little is known about their involvement in acupuncture related effects. Repeated electroconvulsive stimuli increased the NPY-LI and NKA-LI concentrations in the hippocampus and occipital cortex [25,29]. These data are in agreement with experiments of He et al. [14] showing that re-
peated electroconvulsive shocks result in an increase of leu-enkephalin and beta-endorphin-LI in the hippocampus.

The present study was to investigate if changes of NPY-, NKA-, SP-, VIP- and GAL-LI in various parts of rat brain could be demonstrated following acupuncture and physical exercise, taking the method of sacrifice into account.

54 male Sprague-Dawley rats (ALAB Sollentuna, Sweden) 200-220 g were used. They were housed 6/cage at 21°C, with water and food ad lib and a 12-h light/dark cycle. The study was approved by the local Ethics Committee.

Comparison of sacrifice. Two groups (6 + 6) were sacrificed by decapitation (G) or by focused microwave irradiation (MWI), using a microwave system (Metabostat, Gerling Moore, CA, maximal power 5 kW, 2450 MHz) specially built for sacrificing rats; the focused energy exposure time was 2 s [26].

Sensory stimulation and physical exercise. Six groups of 6 rats, anesthetized with chloralhydrate (0.4 g/kg) i.p., received sensory stimulation for 30 min (manual acupuncture, electro-acupuncture or SHAM) once or ten times. The rats were sacrificed by MWI immediately following termination of the treatment. Points chosen for stimulation were UB11 (close to the shoulder joint) and UB54 (close to the hip joint) bilaterally. Stimulation was by rotations of the needles (0.3 mm) after insertion to depths of 0.5–0.7 cm at the points chosen with further rotations repeated for 10 s every 5 min. Electrical stimulation was performed through all 4 needles (UB11–UB11, UB54–UB54) connected in pairs to an acupuncture pulse stimulator (B.V. Enraf-Nonius, Delft, the Netherlands) producing bipolar square wave pulses of 0.2 ms duration and 2 Hz frequency. The current intensity was adjusted so that localized muscle contractions were seen (2–5 V). For SHAM stimulation needles were inserted superficially at the same points and left for 30 min.

One group (6 rats) performed physical exercise (running for 1 h in a wheel (diameter 22 cm and width 9 cm) with a little resistance to turning) and were immediately sacrificed by MWI.

Tissue samples preparation. Following sacrifice the brains were quickly removed, dissected on dry ice into frontal cortex, striatum, occipital cortex, hippocampus, pituitary and hypothalamus [9], weighed and stored at -80°C until extraction. The samples were cut into small pieces in the frozen state, boiled for 10 min in 1 mol/l acetic acid and homogenized. After centrifugation 1000 ×g for 10 min, the supernatants were lyophilized and stored at -40°C before analysis [26].

Radioimmunoassays. The tissue concentrations of NPY-, NKA-, SP-, VIP- and GAL-LI were analyzed by competitive radioimmunoassays. NPY-LI was analyzed using antiserum N1 which cross-reacts 0.1% with avian pancreatic polypeptide, but not with other peptides [27]. The detection limit of the assay was 11 pmol/l. Intra- and interassay coefficients of variation were 7 and 12%, respectively. NKA-LI was analyzed using antiserum K12 which reacts with NKA (100%), NKA (3–10) (48%), neurokinin B (26%), neuropeptide K (61%) and eledoisin (30%) but not with SP [28]. SP-LI was analyzed using antiserum SP2 which reacts with SP and SP sulfoxide, but not with other tachykinins [3]. VIP-LI was analyzed using antiserum VIP2 raised against conjugated natural porcine VIP. The antiserum does not cross-react with gastrin, pancreatic polypeptide, glucagon, neurotensin or NPY. The detection limit of the assay was 3 pmol/l. Intra- and interassay coefficients of variation were 9% and 13%, respectively. GAL-LI was analyzed using antiserum RatGala4 raised against conjugated synthetic rat galanin. The antiserum does not cross-react with NKA, neuropeptide K, SP, NKB, neuropeptide Y, gastrine, pancreatic polypeptide, glucagon or neurotensin. The detection limit of the assay was 5 pmol/l. Intra- and interassay coefficients of variation were 6 and 10%, respectively.

Statistical analyses. Data are presented as means ± S.D. Neuropeptide-LI was first analyzed using multivariate analysis of variance (MANOVA) with SHAM groups as independent variables. When a significant group effect was found, differences in neuropeptide-LI concentrations in each brain region were tested between the groups, using the Kruskal–Wallis test. P < 0.05 were considered significant.

The results of the present study show that the concentrations of SP-LI, NKA-LI and NPY-LI were lower in the brains of guillotined rats (G) than those sacrificed by MWI, but they differed significantly only...
in four brain areas (Fig. 1). Using the mean neuropeptide concentrations of MWI animals as the baseline, the SP-LI was 30% lower in striatum (125 pmol/g (MWI), 88 pmol/g (G)), the NKA-LI concentration 32% lower in the pituitary (135 pmol/g (MWI), 92 pmol/g (G)). The NPY-LI concentrations were 25% and 32% lower in occipital cortex and hippocampus, respectively (125 pmol/g (MWI), 95 pmol/g (G); and 153 pmol/g (MWI), 104 pmol/g (G)). In contrast, no regional differences in VIP-LI and GAL-LI concentrations were observed.

ELACU had an effect on neuropeptide concentrations in three brain regions and moreover, the changes were more prominent following ten procedures (Table 1). VIP-LI concentrations were increased in striatum following one ELACU compared to SHAM (15 ± 3 pmol/g vs. 9 ± 3 pmol/g, P = 0.02). In occipital cortex there was a significant difference in the GAL-LI when comparing one ELACU to one ACU (12 ± 5 pmol/g vs. 6 ± 3 pmol/g, P = 0.02), while only a tendency towards increase was noticed when ELACU was compared to SHAM (12 ± 5 pmol/g vs. 8 ± 4 pmol/g, n.s.) (not shown in the table).

Repeated ELACU significantly elevated NPY-LI concentrations in the hippocampus and occipital cortex; a similar effect was observed for SP-LI and NKA-LI in the hippocampus. NPY-LI concentration following ten ELACU treatments in the hippocampus was 274 ± 57 pmol/g (vs. 123 ± 19, P = 0.004 SHAM and vs. 132 ± 35, P = 0.004 ACU) and in the occipital cortex 154 ± 23 pmol/g (vs. 94 ± 34, P = 0.01 SHAM and vs. 111 ± 29, P = 0.025 ACU). After ten ELACU treatments SP-LI was 27 ± 9 pmol/g (vs. 16 ± 5, P = 0.04 ACU) and NKA-LI was 67 ± 19 pmol/g (vs. 23 ± 15, P = 0.008 ACU and vs. 44 ± 23, P = 0.05, SHAM) in the hippocampus.

Manual acupuncture whether performed once or ten times, and a single 1 h running exercise, had no effect on any of the neuropeptides tested.

Until recently the attention of investigations on mechanisms activated by acupuncture has been focused on endogenous opioids [2]. However, effects on other neurotransmitter systems are also likely. This is supported by studies showing that serotoninergic, dopaminergic and catecholaminergic systems are associated with acupuncture effects [7]. In the present study NPY-LI was found to be increased in the hippocampus and occipital cortex following ten ELACU, while manual stimulation, physical exercise and a single one hour ELACU (unpublished observations) did not bring about any NPY-LI changes in the corresponding brain areas.

The method of sacrifice is important when studying neuropeptides in the rat brain. It has been shown that sacrifice with focused microwave irradiation (MWI) influences the recovery of neuropeptides in whole brain homogenates [26]. In the present study the recovery of neuropeptides was generally higher in the different brain regions of the rats sacrificed with MWI compared to those sacrificed by guillotine. NPY-LI in the occipital cortex and hippocampus as well as NKA-LI in the pituitary and SP-LI in the striatum were significantly lower in the brains of guillotined rats. This and previous studies suggest that MWI is the method of

Table 1

<table>
<thead>
<tr>
<th>Brain Region</th>
<th>SP-LI</th>
<th>NKA-LI</th>
<th>VIP-LI</th>
<th>NPY-LI</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Hippocampus</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 SHAM</td>
<td>19 ± 4</td>
<td>28 ± 11</td>
<td>24 ± 12</td>
<td>154 ± 32</td>
</tr>
<tr>
<td>1 ACU</td>
<td>16 ± 5</td>
<td>29 ± 15</td>
<td>19 ± 4</td>
<td>126 ± 41</td>
</tr>
<tr>
<td>1 ELACU</td>
<td>17 ± 14</td>
<td>26 ± 14</td>
<td>23 ± 6</td>
<td>123 ± 29</td>
</tr>
<tr>
<td>10 SHAM</td>
<td>17 ± 8</td>
<td>44 ± 23</td>
<td>19 ± 5</td>
<td>124 ± 19</td>
</tr>
<tr>
<td>10 ACU</td>
<td>16 ± 5</td>
<td>23 ± 15</td>
<td>21 ± 5</td>
<td>132 ± 35</td>
</tr>
<tr>
<td>10 ELACU</td>
<td>27 ± 9</td>
<td>67 ± 19</td>
<td>17 ± 9</td>
<td>274 ± 57</td>
</tr>
<tr>
<td><strong>Occipital cortex</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 SHAM</td>
<td>18 ± 7</td>
<td>27 ± 19</td>
<td>28 ± 10</td>
<td>126 ± 16</td>
</tr>
<tr>
<td>1 ACU</td>
<td>18 ± 6</td>
<td>23 ± 12</td>
<td>30 ± 7</td>
<td>128 ± 33</td>
</tr>
<tr>
<td>1 ELACU</td>
<td>15 ± 13</td>
<td>24 ± 9</td>
<td>31 ± 15</td>
<td>113 ± 19</td>
</tr>
<tr>
<td>10 SHAM</td>
<td>17 ± 8</td>
<td>21 ± 8</td>
<td>34 ± 18</td>
<td>94 ± 34</td>
</tr>
<tr>
<td>10 ELACU</td>
<td>16 ± 12</td>
<td>19 ± 8</td>
<td>33 ± 11</td>
<td>111 ± 29</td>
</tr>
<tr>
<td>10 ELACU</td>
<td>22 ± 12</td>
<td>27 ± 12</td>
<td>26 ± 11</td>
<td>154 ± 23</td>
</tr>
<tr>
<td><strong>Striatum</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 SHAM</td>
<td>125 ± 32</td>
<td>139 ± 41</td>
<td>9 ± 3</td>
<td>187 ± 98</td>
</tr>
<tr>
<td>1 ACU</td>
<td>115 ± 43</td>
<td>152 ± 46</td>
<td>13 ± 3</td>
<td>197 ± 96</td>
</tr>
<tr>
<td>1 ELACU</td>
<td>110 ± 36</td>
<td>145 ± 29</td>
<td>15 ± 3</td>
<td>188 ± 34</td>
</tr>
<tr>
<td>10 SHAM</td>
<td>117 ± 40</td>
<td>138 ± 36</td>
<td>18 ± 8</td>
<td>183 ± 72</td>
</tr>
<tr>
<td>10 ACU</td>
<td>120 ± 16</td>
<td>154 ± 35</td>
<td>13 ± 6</td>
<td>187 ± 71</td>
</tr>
<tr>
<td>10 ELACU</td>
<td>116 ± 35</td>
<td>101 ± 22</td>
<td>11 ± 7</td>
<td>156 ± 35</td>
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</table>

Values are expressed as means ± S.D. for six animals. * P < 0.05, ** P < 0.01.
choice of sacrifice when studying neuropeptides in the rat brain.

Of the areas studied the hippocampus and the occipital cortex showed the most pronounced changes in neuropeptide concentrations following repeated ELACU. In the present study a significant increase of NPY-LI was seen only following ten ELACU treatments. It has been shown that under conditions of negative energy balance, such as starvation and intense exercise [5,18], there is a regional rise in hypothalamic NPY concentrations. NPY injected into the paraventricular nucleus stimulates insulin and glucagon secretion [1], ACTH and corticosterone release [31]. Through increased activity and release, NPY may serve a homeostatic function in defending body weight by stimulating feeding and energy expenditure [33]. This is interesting to note as acupuncture is thought to 'regulate energy homeostasis' in classical Chinese medicine. Furthermore, since the serotonin-containing nerve endings terminate on NPY-synthesizing cells [10], NPY and serotonin may interact functionally to control energy homeostasis [8], with serotonergic systems playing an inhibitory role in the regulation of NPY [16].

In experimental and clinical studies it has also been shown that NPY may play a role in psychiatric disorders, particularly depression, which is claimed to involve a deficit of central NPY [30]. Depressed patients exhibit reduced concentrations of NPY-LI in CSF [32]. In an animal model of depression, reduced NPY-LI concentrations were found in the cerebral cortex [32], while treatment of rats with antidepressants [15,32] or repeated electroconvulsive stimuli [25] increased cortical concentrations of NPY-LI. Interestingly, it has been reported that acupuncture is as effective as antidepressants in the treatment of depressive disorders [11]. Our observations indicate that repeated ELACU treatments increase the concentrations of NPY-LI in the hippocampus and occipital cortex. In view of the relatively slow changes seen, electro-acupuncture mostly probably alters the synthesis, processing or metabolism of NPY or its precursor protein, rather than causing its local release or breakdown.

An increase of NKA-LI and SP-LI concentrations is of interest as the same effect was obtained in the frontal cortex in rats after antidepressive treatment using selective serotonin uptake blockers [4].

In conclusion, the main results presented in this study show that repeated electro-acupuncture treatments result in significantly increased concentrations of rat brain NPY-, NKA- and SP-LI primarily in the occipital cortex and hippocampus. The functional significance of this increase needs further investigations.

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[17] Kashiba, H. and Ueda, Y., Acupuncture to the skin induces release of substance P and calcitonin gene-related peptide from


