Effects of Repeated Sensory Stimulation (Electro-acupuncture) and Physical Exercise (Running) on Open-field Behaviour and Concentrations of Neuropeptides in the Hippocampus in WKY and SHR rats

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Abstract

The effects of repeated sensory stimulation (electro-acupuncture) and physical exercise (running) on open-field behaviour and on hippocampal concentrations of neuropeptide Y, neurokinin A, substance P, galanin and vasoactive intestinal peptide (VIP)-like immunoreactivities were studied in WKY (Wistar-Kyoto) and SHR (spontaneously hypertensive) rats. Significantly higher concentrations of substance P-like immunoreactivity, neurokinin A-like immunoreactivity and neuropeptide Y-like immunoreactivity were found in the hippocampus immediately after 3 weeks of treatment (electro-acupuncture and running), but not 1 week after the last (tenth) intervention. No changes in galanin-like immunoreactivity and VIP-like immunoreactivity were found. The changes in neuropeptide concentrations were similar in the two rat strains. Open-field behaviour was significantly reduced during the treatment period in both strains. There were significant negative correlations between behaviour and neuropeptide concentrations in SHR rats, suggesting interdependency with sympathetic activity. It is proposed that the effects of electro-acupuncture and physical exercise in rats are related to increases in neuropeptide Y, neurokinin A and substance P in the hippocampus.

Introduction

Repeated electro-acupuncture has an antidepressive effect in humans (Han, 1986; Liu et al., 1992), and activates numerous peptidergic systems in the central nervous system (Debreceni, 1993). Repeated but not single electro-acupuncture treatments have produced increased concentrations of neuropeptide Y (NPY), neurokinin A (NKA) and substance P-like immunoreactivity (LI) in the rat hippocampus and occipital cortex (Bucinskaite et al., 1994). The present experiments were carried out to investigate if the effects of electro-acupuncture on behaviour correlate with any changes in neuropeptide concentrations in two rat strains with differing sympathetic activity. Since it has been suggested that ergo-receptors (Knuffki et al., 1981) and afferent nerve fibres are similarly activated by electro-acupuncture and physical exercise (Andersson, 1993), we compared these two modes of somatic afferent stimulation.

The Wistar-Kyoto (WKY) rat strain, with its normal blood pressure, is often selected as the control in experiments performed on spontaneously hypertensive (SHR) rats (Okamoto, 1969; Kurtz and Moms, 1987). In contrast to SHR rats, WKY rats show less spontaneous activity (Knardahl and Sagvolden, 1979) and mobility in the open-field test (Hård et al., 1985). Reduction in spontaneous motor activity is widely accepted as a sensitive measure of the sedative effects of drugs on animals. It may result from noradrenaline or serotonin reuptake inhibitors, noradrenergic agonists, mixed receptor blockers, or drugs possessing a wide range of neurochemical effects (such as tricyclics) (Tucker and File, 1986). It therefore seems unlikely that a single neurochemical mechanism mediates the behavioural changes (Stone, 1983). Besides the classical transmitters, NPY seems to play a role in motor behaviour. NPY (Tatemoto et al., 1982) coexists and is coreleased with noradrenaline from sympathetic nerve endings (Lundberg and Hökfelt, 1983; Everitt and Hökfelt, 1989) during major activation of the sympathetic nervous system (Rorie et al., 1990). Centrally administered NPY suppresses locomotor activity in a dose-related (and reversible) manner (Heilig and Murison, 1987), and induces EEG changes characteristic of sedation (Fuxe et al., 1983). Anxiolytic effects of centrally administered NPY are mediated, at least in part,
through NPY Y1 receptors in the amygdala (Heilig et al., 1993). This effect can be blocked by the α2 adrenergic antagonist idazoxan, suggesting an interaction with the noradrenergic system (Heilig et al., 1989). On the basis of NPY action in memory-related behavioural assays (Flood et al., 1987) and with the high concentration in the hippocampus and amygdala taken into account (Gray and Morley, 1986), it is suggested that NPY may play a role in memory modulation. Also, the retrohippocampal region, exceptionally rich in NPY-LI axons, includes NPY-positive afferents originating from the lateral nucleus of the amygdala (Köhler et al., 1986).

Substance P and NKA, belonging to the tachykinin family of peptides, are cosynthesized and coreleased in some brain regions (Dalsgaard et al., 1985; Lindefors et al., 1985). A distinct, well organized substance P innervation is observed in the hippocampal formation (Vincent et al., 1981). Both serotonin (5-HT) and noradrenaline (Storm-Mathisen and Guldberg, 1974) are found in high concentrations in the hippocampus. Substance P alters the metabolism and release of brain dopamine and 5-HT (Magnusson et al., 1976; Hall et al., 1987), while the biosynthesis of tachykinins is controlled by dopamine receptor-mediated mechanisms (Bannon et al., 1987). Antidepressive treatment using selective serotonin uptake blockers significantly increases the concentrations of substance P and NKA in a dose-dependent way in the frontal cortex in rats (Brodin et al., 1987). It is suggested that the effect of serotonin uptake inhibitors is mediated not only by the turnover of monoamines but also by a changed turnover of coexisting substance P (Bartfai, 1985).

Materials and methods

Ninety male SHR and 90 male WKY rats (Mollegaard, Denmark), weighing 200–220 g at the beginning of the experiments, were used. They were housed six to a cage with water and food ad libitum, ambient temperature 21°C and a 12 h light/dark cycle.

Animals of both strains were randomly divided into three groups. Two groups each of 30 WKY and 30 SHR rats were anaesthetized with chloral hydrate (0.4 g/kg i.p.) and given sensory stimulation (electro-acupuncture or sham) for 30 min twice a week from the third to the eighth week of the experiment (Fig. 1). The running wheels were constructed with ball-bearings to minimize resistance to turning (Shyu et al., 1984). When not loaded by the weight of the rat the wheel was locked by a stopper to prevent erroneous counts of actual voluntary running activity (no force was used to teach the rats to run). The number of revolutions of the wheel, during the 30 min running period, was measured by an electromagnet and recorded at the end of experiments. The median distance during the 30 min running period was 0.17 km for SHR and 0.12 km for WKY rats at the start of the experiment and 0.26 and 0.19 km respectively at the tenth session. The day previous to running they were given the same amount of anaesthetic as the electro-acupuncture and sham-stimulated groups.

Behavioural tests were performed 6 h after each treatment. All tests were conducted in the laboratory room during the light portion of the light/dark cycle and the behaviour of every rat was observed for 9 min.

Open-field behaviour

The experiment was conducted in an open-field apparatus (Köhler et al., 1978) measuring 96 × 96 × 56 cm. The floor was divided into 15 × 15 cm sections by lines (1 cm wide) of black paint. In the centre there was a 25 × 41 cm hole where the rat's home cage was placed. The top of the home cage was 3 cm above the floor of the open field. The following types of behaviour were recorded: activity in the home cage (number of enterings/leavings of the home cage with all four paws), 'middle crossings' (number of times the animal crossed the section located just outside the home cage with all four paws) and 'outer crossings' (number of crossings of the sections located next to the outer walls of the open-field apparatus). The total ambulatory activity was calculated as the sum of home cage activity, middle and outer crossings (Knardahl and Sagvolden, 1979). All the behavioural tests were performed by an observer ignorant of the preceding treatment.

Tissue collection

Ten animals from each group were killed after 5, 8 and 10 weeks, on the day following the last behavioural experiment, by focused microwave irradiation. A specially built microwave system (Metabostat, Gerling Moore, CA; maximal power 5 kW, 2450 MHz) with focused energy exposure time 2 s (Theodorsson et al., 1990) was used. The brain of each animal was quickly removed and dissected on dry ice (Glowinski and Iversen, 1966), and the hippocampus was weighed and stored at −80°C until extraction.

The samples were cut into small pieces while frozen, boiled for 10 min in 1 mol/l acetic acid and homogenized. After centrifugation at 1000 g for 10 min, the supernatants were lyophilized and stored at −20°C before analysis.

Radioimmunoassays

The tissue concentrations of NPY-, NKA-, substance P-, VIP- and galanin-LI were analysed by competitive radioimmunoassays. NPY-LI was analysed using antiseraum N1, which cross-reacts 0.1% with avian pancreatic polypeptide but not with other peptides (Theodorsson-Norheim et al., 1985a). The detection limit of the assay was 11 pmol/
Intra- and interassay coefficients of variation were 7 and 12% respectively. NKA-LI was analysed using antiserum K12, which reacts with NKA (100%), NKA(3-10) (48%), neurokinin B (26%), neuropeptide K (61%) and eldoisoin (30%), but not with substance P (Theodorsson-Norheim et al., 1985b). Substance P-LI was analysed using antiserum substance P2, which reacts with substance P and substance P sulphoxide but not with other tachykinins (Brodin et al., 1986). VIP-LI was analysed using antiserum VIP2 raised against conjugated natural porcine VIP. The antiserum does not cross-react with gastrin, pancreatic polypeptide, glucagon, NPY or neurotensin (E. Theodorsson, unpublished). The detection limit of the assay was 3 pmol/l. Intra- and interassay coefficients of variation were 9 and 13% respectively. Galanin-LI was analysed using antiserum RatGala4 raised against conjugated synthetic rat galanin. The antiserum does not cross-react with NKA, neuropeptide K, substance P, NKB, NPY, gastrin, pancreatic polypeptide, glucagon or neurotensin. HPLC-purified \(^{125}\text{I}\)-rat galanin was used as radioligand and rat galanin as a standard. The detection limit of the assay was 5 pmol/l. Intra- and interassay coefficients of variation were 6 and 10% respectively (E. Theodorsson, unpublished).

**Statistical analysis**

Neuropeptides were first analysed using multivariate analysis of variance, with treatment and time as independent variables. When a significant group effect was found, differences in every neuropeptide concentration between the groups were tested using the Tukey test; \(P < 0.05\) was considered significant.

Open-field behaviour was analysed using three-way analysis of variance, with treatment as independent and time as dependent measure, using the behavioural scores for the 5 weeks during which

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**Fig. 2.** Concentrations of neuropeptides (pmol/g wet weight) found in WKY and SHR rat hippocampus after sham stimulation, sensory stimulation by electroacupuncture (ELACU) or running, performed twice a week. Numbers under bottom panel refer to all panels: 3 indicates after 3 weeks of treatment, +1 indicates 1 week after the final treatment, and +3 indicates 3 weeks after the final treatment. Values are mean ± SD for ten animals. \(*P < 0.05; **P < 0.01; ***P < 0.001.\)
treatment was performed. Then the effects of treatment were compared to results for sham-treated groups using the Tukey test.

The Spearman correlation between each neuropeptide and open-field behaviour was calculated pairwise for every treatment. Significance of the correlation coefficient was tested using the t-test (two-tailed).

Results

**Neuropeptide concentrations in hippocampus**

Three-way analysis of variance considering strain × treatment × time showed no main effect of strain in strain × treatment, strain × time or strain × treatment × time interaction effects. The main effect of time was significant for all five neuropeptides (P < 0.005). The main effect of treatment was also significant for all neuropeptides (P < 0.05) except VIP-LI. The interaction treatment × time had a significant main effect on NPY-LI, NKA-LI and substance P-LI (P < 0.001).

Many similarities in neuropeptide concentrations were found in both WKY and SHR strains when analysed separately using two-way ANOVA. In WKY rats the interaction of treatment × time had a significant main effect for NPY-LI (F(4,481) = 13.14, P < 0.001), NKA-LI (F(4,481) = 5.99, P < 0.001), substance P-LI (F(4,481) = 11.93, P < 0.001) but no main effect on galanin-LI or VIP-LI. Using the Tukey test a significant increase was found following 3 weeks of repeated sensory stimulation/physical exercise (Fig. 1). NPY-LI was elevated from 16.8 ± 4.8 to 29.6 ± 8.0 and 32.7 ± 7.9 pmol/g, P < 0.001; NKA-LI from 13.3 ± 3.7 to 23.2 ± 8.7 and 23.1 ± 6.6 pmol/g, P < 0.001; and substance P-LI from 7.2 ± 2.9 to 17.4 ± 6.2 and 13.5 ± 5.0 pmol/g, P < 0.001 and P < 0.01 respectively. No changes were observed at the later time points.

In the SHR rats, treatment × time interaction had a significant main effect for NPY-LI (F(4,83) = 13.79, P < 0.001), NKA-LI (F(4,83) = 8.82, P < 0.001) and substance P-LI (F(4,83) = 8.7, P < 0.001), while no effect on galanin-LI or VIP-LI was observed. Using the Tukey test a significant increase in NPY-LI in the hippocampus (Fig. 2) was found following 3 weeks of repeated sensory stimulation or physical exercise (twice a week) compared with controls (30.3 ± 7.8 and 32.7 ± 8.3 pmol/g versus 17.9 ± 5.0, P < 0.001). One week after the last sensory stimulation the concentration of NPY-LI was decreased compared with the control group (11.1 ± 3.5 pmol/g versus 18.9 ± 4.4, P < 0.05). No changes were found 3 weeks after the last procedure. NKA-LI and substance P-LI were elevated 3 weeks after sensory stimulation or running (NKA-LI, 23.3 ± 6.9 and 23.9 ± 7.9 pmol/g versus 13.0 ± 4.1, P < 0.001; substance P-LI, 16.2 ± 5.5 and 13.5 ± 5.9 pmol/g versus 6.7 ± 2.6, P < 0.001 and P < 0.01 respectively). NKA-LI and substance P-LI did not differ from the controls when sensory stimulation or running were finished.

**Total ambulatory activity**

There was a statistically significant main effect of strain (F(1,114) = 308.2, P < 0.001) and treatment (F(2,114) = 88.18, P < 0.001) as well as a significant interaction of strain × treatment (F(2,114) = 24.68, P < 0.001), strain × time (F(4,456) = 3.46, P < 0.01) and treatment × time (F(8,456) = 2.04, P < 0.05).

In the WKY rat strain both electro-acupuncture and running reduced the total ambulatory activity, but this reduction failed to reach statistical significance at any time during the 5 week treatment period (Fig. 3A). In the SHR rat strain (Fig. 3B) electro-acupuncture reduced total ambulatory activity after 1 week of treatment (P < 0.05) and it remained reduced for another 4 weeks (P < 0.001), returning to the control level 1 week after the treatment. The effect of running made a significant difference after 2 weeks (P < 0.001), which remained at the same significant level until the end of treatment but did not differ from sham- or electro-acupuncture-treated groups after the treatment was finished.

There was no difference in the effects of electro-acupuncture treatment or running in either strain at any period of analysis.

**Correlations between hippocampal neuropeptide concentrations and open-field behaviour**

Spearman correlation coefficients were calculated between individual concentrations of neuropeptides in the hippocampus and open-field behaviour scores.

In the SHR strain there was a significant negative correlation between substance P-LI (-0.388; P < 0.05, two-tailed test), NKA-LI (-0.379; P < 0.05) and NPY-LI (-0.541; P < 0.01) and open-field behaviour in the exercising group. The group treated with electro-acupuncture showed a significant negative correlation between NKA-LI and open-field behaviour (-0.431; P < 0.05).
In the WKY strain no significant correlations were found between any neuropeptide-like immunoreactivity and open-field behaviour.

Discussion

Acupuncture is part of traditional Chinese medicine, a system with an empirical basis which has been used in the treatment and prevention of disease for centuries. A lack of scientific studies to prove or disprove its claimed effects led to its rejection by many in the Western scientific community. Now many of its mechanisms can be partly explained in scientific terms; for example, its use for pain relief has been supported by clinical trials (Thomas, 1995).

The effects of acupuncture must devolve from physiological and/or psychological mechanisms, and needle stimulation could represent the artificial activation of systems normally stimulated by natural biological effects in functional situations. Recent studies have shown that acupuncture and some other forms of sensory stimulation elicit similar effects in man and other mammals (Andersson and Lundeberg, 1995), suggesting that they bring about fundamental physiological changes. Acupuncture excites receptors or nerve fibres in the stimulated tissue which are also physiologically activated by strong muscle contractions (Chang et al., 1973). The effects on certain organ functions are possibly similar to those obtained by protracted exercise. Both exercise and acupuncture, especially electro-acupuncture, produce rhythmic discharges in nerve fibres (Andersson et al., 1973). Further, both exercise and acupuncture cause the release of endogenous neurotransmitters (Debreceni, 1993) essential to the induction of functional changes in different organ systems and behaviour (Andersson and Lundeberg, 1995).

In the present study both repeated electro-acupuncture treatment and physical exercise increased concentrations of NPY-, NKA- and substance P-LI in the hippocampus in the two rat strains despite their differing sympathetic activity.

Our working hypothesis was that the regional brain tissue concentrations of neuropeptides should reflect changes in motor and sympathetic activity (Heilig and Murison, 1987; Heilig et al., 1989). We found similar concentrations of peptides in the two control groups, with similar increases in NPY-, substance P- and NKA-LI concentrations. No changes in VIP- and galanin-LI were seen in either SHR or WKY rats after repeated electro-acupuncture or running. Our data for the control groups were in line with a previous report (Maccrernone and Jarrott, 1985), where no differences were found in NPY-LI in hippocampus and hypothalamus between the two strains. Our data showed that repeated electro-acupuncture altered neuropeptide concentrations in the hippocampus, the region of the rat brain expressing the highest concentrations of $^{125}\text{I}$NPY binding sites (Chang et al., 1985). In addition, the duration of NPY effect on monoamine turnover is longer in the hippocampus than in any other rat brain area (Vallejo et al., 1987). The correlation between the increased concentration of NPY-LI and suppressed locomotor activity is in line with the data of Heilig and Murison (1987), who attribute the activity-suppressing effect of NPY to centrally mediated behavioural sedation.

Microinjections of substance P, NKA and related tachykinins and selective NK1 agonists into the ventral tegmental area or the nucleus accumbens increase the local release of dopamine (Kalivas and Miller, 1984) and elevate locomotor activity (Elliott et al., 1992; Elliott and Iversen, 1986). NKA being ten times more potent than substance P (Kalivas et al., 1985). The reasons why increased concentrations of tachykinins ran in parallel with decreased locomotor activity in our experiments might include cross-desensitization to tachykinin-induced effects. Substance P and NKA differ considerably in their ability to induce desensitization. While substance P very potently desensitizes its own cardiovascular and behavioural responses and those to NKA, the ability of NKA to desensitize its own cardiovascular and behavioural effects or those of substance P is less pronounced (Culman et al., 1993).

The data obtained support the idea that one of the factors determining the decrease in open-field activity in rats following electro-acupuncture or running is an increase in NPY-, substance P- and NKA-LI in the hippocampus. It is possible that these changes play an important role in the antidepressive effects of acupuncture and physical exercise. Experimental and clinical studies suggest that afferent input in somatic nerve fibres has a significant effect on autonomic functions. Hypothetically, the physiological counterpart lies in physical exercise, and the effect can be artificially reproduced via various types of electrical or manual stimulation of certain nerve fibres.

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Abbreviations

- LI -like immunoreactivity
- NKA neurokinin A
- NPY neuropeptide Y
- SHR spontaneously hypertensive rat
- VIP vasoactive intestinal peptide
- WKY Wistar-Kyoto

References

Chang, R. S. L., Lotti, V. J., Chen, T.-B., Cerino, D. J. and Kling, P. J. (1985) Neuropeptide Y (Y) binding sites in rat brain labeled with $^{125}$I-Bolton-


