

THE AMELIORATION OF RESTRAINT STRESS BY ELECTROSTIMULATION

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Neuro-Electric Therapy (NET) has been described previously (1) as being effective in the amelioration of the withdrawal stress symptoms of drug addiction and alcoholism. NET is an electro-stimulation technique in which cutaneous electrodes are placed over the human mastoid area and a milliampere alternating current is passed, at varying frequencies and pulse widths, for variable periods of time. The present experiment was designed to investigate the biochemical basis by which NET ameliorates stress in experimental animals.

Table 1: The effect of NET on the plasma cortisol of rats subjected to restraining stress.

Animal	Cortisol* Mean \pm SD	P
NET treated		
1	56	
2	101	
3	100	
4	30	
5	94	
6	39	70 ± 32 < 0.001
Sham treated		
1	134	
2	170	
3	143	
4	178	
5	185	
6	—	162 ± 22

*Measured in ng/ml. One animal was eliminated from the Sham treated group as it suffered an intercurrent illness during the experiment.

Methods: Male Sprague-Dawley rats of body weight 250 ± 20 g were housed 6 per cage in standard high density polypropylene cages on sawdust bedding. Two groups of 6 rats had each ear pierced by insertion of a Michel suture clip, which was used as a percutaneous electrode because cutaneous electrodes were found to be impracticable in experimental animals. Twentyfour hours after insertion of the ear clip the rats were placed in individual (Boltzmann-type) restraining cages. Cages containing rats for NET and sham treatment were placed alternately in close proximity on a bench. The electric current was passed from the NET apparatus through the earclip 'electrodes' via crocodile clamps at the terminus of each NET apparatus. The electrical parameters selected are those used by other workers in the electro-stimulation field (2). The sham treated animals were connected via their electrodes to wires but no electricity was passed. Both sets of animals remained in their restraining cages for 3 hours during which the ambient temperature was kept constant (21°C) and all other stressful factors, e.g. noise, were kept to a minimum. The NET parameters were kept constant; frequency 100 Hz; pulse width 0.22 msec; mean voltage for 6 animals 9–11 v.

Table 2: The levels of some hepatic microsomal enzymes in NET and sham treated rats subjected to restraint stress for 3 hours.

Group	Animals (no.)	Average body weight (g)	Mean liver weight (g)	Average microsomal protein (mg/g)	Cytochrome P ₄₅₀ *	Aryl hydrocarbon hydroxylase †	Nitroanisole demethylase ‡	UDPGA transferase †
Sham	6	250 ± 20	10.8 ± 0.61	30.8 ± 3.4	0.95 ± 0.27	0.09 ± 0.05	0.28 ± 0.10	1.03 ± 0.30
NET	6	250 ± 20	10.6 ± 0.41	31.2 ± 1.7	1.1 ± 0.10	0.34 ± 0.12^a	0.36 ± 0.05^b	1.44 ± 0.17^c

Results represent the mean \pm SD for the number of animals indicated. *nmol/mg protein, †nmol/mg protein/min. Significant differences from Sham P < 0.001, ^a0.05 > 0.02, ^b0.05 > 0.02.

After treatment the animals were sacrificed by cervical fracture, exsanguinated by cardiac puncture, and their livers surgically excised. The plasma was separated by centrifugation, and cortisol determined by radioimmunoassay (3). The livers were homogenised in ice-cold KCl (1.15%) and microsomes were prepared by centrifugation in the manner described previously (4). Cytochrome P₄₅₀, aryl hydrocarbon hydroxylase (AHH) and uridine diphosphoglucuronic acid (UDPGA) transferase were all estimated in the manner previously described (4). *p*-Nitroanisole demethylase was measured by the technique of Moldeus *et al* (5). The significance between observations on NET treated and sham treated animals was determined by unpaired Student's *t* test. Differences were considered significant at $P < 0.05$.

Results: The plasma cortisol levels were significantly lower ($P < 0.001$) in the NET treated rats (table 1). The effect of NET on the hepatic parameters investigated is given in table 2. There is no significant change in liver weight or microsomal protein in either NET or sham treated rats. The cytochrome P₄₅₀ levels of NET treated rats were similar to those of control animals, whereas the sham treated levels were variable, most were significantly lower than the expected value. The activities of AHH, nitroanisole demethylase and UDPGA transferase of the NET treated animals were significantly higher than those of the sham treated controls; for AHH $P < 0.001$, nitroanisole demethylase $P < 0.05 > 0.02$, and UDPGA transferase $P < 0.05 > 0.02$. Thus, the decreased cortisol levels of the NET treated animals suggest that electro-stimulation has an ameliorating effect on the stress-treatment the animals received. Stress (and cortisol) are stimulators of mixed function oxidase enzyme activity (6). Paradoxically, in the present experiment the activity of the mixed function oxidases was increased by electrostimulation despite the decreased cortisol levels. Therefore, further work is necessary to elucidate the mechanism(s) by which NET enhances hepatic enzyme activity.

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