

TRYPTOPHAN, SEROTONIN AND HYDROXYINDOLE ACETIC ACID LEVELS IN RAT BRAIN FOLLOWING SLOW OR FAST FREQUENCY ELECTROSTIMULATION

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The technique of therapeutic electrostimulation (ES) has developed from acupuncture and is becoming increasingly used for the control of pain (1) and the amelioration of the symptoms associated with the withdrawal of drugs of abuse from addicted subjects (2). The lack of experimental models with which to correlate biochemical observations impedes studies designed to identify the most important factors in the mechanisms of action of ES (3). ES has been demonstrated to decrease the duration of the loss of righting reflex (LRR) in animals anaesthetized following administration of acute doses of narcotic substances (4, 5). Using this as a model system the differences between various forms of ES reducing LRR have been described (5). Previous reports have correlated the amelioration of pain elicited by ES via electrodes chronically implanted in the periaqueductal grey or median raphe nucleus with localised alterations in serotonin (5HT) metabolism (6). In the present study, therefore, the levels of 5HT, its precursor, tryptophan (TRP), and its main metabolite, hydroxyindole acetic acid (HIAA), were determined in various regions of the brains of rats which had had their sleeping time significantly reduced by transcutaneous electrostimulation.

Methods: Female CD rats of body weight 250 ± 20 g were purchased from Charles River, Manston, Kent. These animals were maintained in groups of six on sterilized sawdust bedding in high density polypropylene cages on a 12 h 8.00–20.00 h light-dark cycle. Food, R. & M. Experimental No. 1 (BP Nutritional) and water were available *ad libitum*. All experiments were conducted between 9.00 and 12.00 h. Rats, in groups of six, were anaesthetized by intraperitoneal administration of hexobarbital (80 mg/2 ml/kg) dissolved in sterile alkaline solution (i.e., the minimal quantity of 5M NaOH solution was added dropwise to the hexobarbital in sterile water until the barbiturate dissolved). Suture clips were inserted into the auditory meatus of the external pinna of each rat to serve as electrodes and these were connected to the termini of the electrostimulators via crocodile clips. A current was applied using an NET (NeuroElectric Therapy) electrostimulator (European Electronic Systems, Maldon, Essex). The applied current was a direct, square wave of pulse width 0.22 msec and frequency either 10 Hz or 500 Hz. The current was continuously monitored across the termini on an oscilloscope. Each stimulator was adjusted until an identical current, of potential difference 1 V with the animal *in situ*, was recorded on the oscilloscope. The treated rats were stimulated continuously, one group of 6 at frequency 10 Hz and another group at 500 Hz, until they regained their righting reflex (5). Sham-treated rats were connected to the stimulators but no current was passed. Immediately after the rats regained their righting reflex they were decapitated, the brains were swiftly excised, and whilst chilled on ice dissected into the seven regions indicated in the table, in the manner described by Glowinski and Iversen (7). The TRP, 5HT and HIAA content of the various regions of the brain were determined by fluorimetric techniques previously described (8, 9) after separating these substances by the column method of Kemerer *et al.* (10). Statistical comparisons between TRP, 5HT or HIAA levels in the brain derived from ES and sham-treated rats were made by unpaired Student's *t* test.

Results: The results in the table demonstrate that ES at either 10 or 500 Hz significantly decrease the LRR duration induced by acute hexobarbital administration. With the exception of the hippocampus, mid-brain and striatum, there was no significant difference between the TRP levels of control or electrostimulated rats. ES at 10 and 500 Hz increased the level and therefore, presumably, the uptake of this neurotransmitter precursor in these brain regions. There was no significant difference between the 5HT levels in the stimulated and sham-treated animals. ES at both frequencies, but in particular slow (10 Hz) frequency, increases HIAA levels in the hippocampus and hypothalamus.

The levels (ng/g wet weight of tissue) of tryptophan, serotonin and hydroxyindole acetic acid in differing brain regions of control and electrostimulated rats (mean \pm SD, n = 6)

Group	Duration of LRR (mins)	Substance	Medulla	Cerebellum	Cortex	Hypothalamus	Hippocampus	Striatum	Mid-brain
Control	90 \pm 11	TRP	1161 \pm 182	1550 \pm 201	1160 \pm 292	4180 \pm 407	1753 \pm 242	1420 \pm 203	660 \pm 72
		5HT	898 \pm 333	215 \pm 45	218 \pm 64	1282 \pm 196	621 \pm 96	489 \pm 103	496 \pm 79
		HIAA	621 \pm 85	115 \pm 14	246 \pm 83	773 \pm 110	300 \pm 48	511 \pm 62	707 \pm 95
ES 10 Hz	48 \pm 8	TRP	1280 \pm 102	1451 \pm 330	910 \pm 114	4270 \pm 386	7241 \pm 657*	2044 \pm 130*	1579 \pm 197*
		5HT	602 \pm 104	147 \pm 31	257 \pm 71	1179 \pm 188	771 \pm 111	353 \pm 76	656 \pm 94
		HIAA	715 \pm 134	151 \pm 34	187 \pm 52	2374 \pm 263*	5612 \pm 637*	766 \pm 117	640 \pm 80
ES 500 Hz	50 \pm 8	TRP	1381 \pm 191	1350 \pm 202	1225 \pm 153	3882 \pm 331	7530 \pm 533*	2450 \pm 221*	1607 \pm 301*
		5HT	706 \pm 141	190 \pm 53	201 \pm 47	943 \pm 213	592 \pm 74	372 \pm 83	515 \pm 86
		HIAA	814 \pm 102	119 \pm 27	338 \pm 57	1226 \pm 153*	1137 \pm 109*	619 \pm 88	621 \pm 71

*Significantly different from the control value $P < 0.05$.

Discussion: The results of the present study confirm previous reports that ES decreases the duration of hexobarbital-induced LRR (4, 5). Chronic ES via surgically implanted electrodes has been demonstrated to increase 5HT synthesis and HIAA levels particularly in the hippocampus (6, 11). It has been suggested that the hippocampus is important in mediating

the effects of 5HT on locomotion (12) and that serotonergic neurons participate in the hypothalamic regulation of anterior pituitary functions (13). Transcutaneous electrostimulation, as employed in the present experiment, also invoked elevations in HIAA levels in these brain regions. 5HT is strongly implicated in the development of tolerance to drugs and their behavioural effects, including hypnosis (14). Increased levels of 5HT are normally associated with a prolongation of sleeping time (LRR) after administration of hypnotic agents (15). Although in the present experiment the 5HT remain constant, the increased TRP and HIAA levels in the hippocampus and hypothalamus are interpreted as indicating increased 5HT synthesis and catabolism.

Barbiturates decrease the activity of 5HT neurons and reduce the turnover of this neurotransmitter in the brain (15). Drugs such as disulfirum, which inhibit 5HT metabolism, potentiate barbiturate-induced LRR, thus prolonging sleeping time (15). The results of the present experiment suggest therefore that the origin of the decrease in narcosis induced by ES of both frequencies could be a function of increased 5HT metabolism in certain areas of the brain.

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