

The Effect of Electrostimulation on Barbiturate-Induced Sleeping Times in Rats

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ABSTRACT

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Female rats, anaesthetized with hexobarbital, regained their righting reflex more rapidly following electrostimulation than sham-treated controls. The extent of the decreased sleeping times in these animals varied according to the frequency (cycles per second) of the electrostimulation applied. The frequency which produced the largest decrease in sleeping time was 10 Hz. Determination of the activity of some microsomal enzymes indicated that the decreased sleeping time was not the result of increased hepatic enzyme activity. Animals which had received prior treatment with naloxone exhibited increased sleeping times following barbiturate administration, but the effects of electrostimulation on the sleeping time at 10 Hz was diminished, while the effect of electrostimulation at high frequency (500 Hz) was enhanced. Although repeated daily administration of hexobarbital progressively decreased sleeping times for all the animals, electrostimulation decreased the sleeping times of the treated rats by a similar percentage of the control animals on each successive day. Electrostimulation at a frequency of 10 Hz produced a significant decrease in serum corticosterone levels, whereas 500 Hz resulted in an increase.

Key words: electrostimulation, frequency, barbiturates, sleeping times, rats, hepatic enzymes

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INTRODUCTION

The practice of acupuncture has been superseded in the West by electroacupuncture, which is presently the subject of intensive experimental research to exploit the phenomenon of "autoanalgesia" (antinociceptive effect). The needles associated with acupuncture may be replaced in clinical practice by electrodes which are placed on the surface of the skin (Heidl et al., 1979; Woolf, 1979).

Neuroelectric therapy (NET) is an electrostimulation technique which involves placing cutaneous electrodes over the human mastoid areas and passing a milliampere current at specific frequencies and pulse widths for a given interval of time [Patterson, 1976]. The majority of previous experimental reports examine various electrical stimulatory parameters and their influence on the analgesic effects produced [Cheng and Pomeranz, 1979, 1980; Heidl et al., 1979; Woolf, 1979]. The stimulatory apparatus (NET) employed in the present experiment has been demonstrated to ameliorate some of the biochemical effects resulting from restraint stress [Capel et al., 1979]. Clinically, this form of electrostimulation (NET) is being successfully employed to alleviate the withdrawal symptoms associated with the treatment of patients addicted to narcotic drugs and alcohol [Patterson, 1976]. This study was conducted, therefore, to investigate the biochemical basis by which electrostimulation might influence drug detoxication. The time taken to regain righting reflex following barbiturate anaesthesia (sleeping time) may provide an *in vivo* estimate of hepatic function. Thus, the effect of electrostimulation at various frequencies on barbiturate-induced sleeping times was monitored to determine whether this treatment influences drug detoxication by altering hepatic function and whether the frequency at which the current is applied influences the result.

The specific narcotic antagonist, naloxone, has been demonstrated to prevent the analgesic response induced by peripheral electrostimulation [Buckett, 1979; Cheng and Pomeranz, 1980]. The hyperalgesia resulting from pretreatment with naloxone is generally regarded as indicative that endorphins have mediated the tolerance to the nociceptive stimulus [Cheng and Pomeranz, 1979]. Naloxone was administered prior to electrostimulation in this study, therefore, to determine whether the observed effects on barbiturate anaesthesia was mediated via these neuropeptides.

MATERIALS AND METHODS

Chemicals

Narcan[®] (naloxone) and Tuinal[®] (equal quantities of quinalbarbitone/amylobarbitone) were obtained from Boots, Nottingham, Notts., U.K. Evipan Natrium[®] (soluble hexobarbital) was a generous gift from Bayer, Haywards Heath, Sussex, U.K. Pentothal[®] (thiopentone, sodium) was purchased from Abbot's Laboratories, Romford, Essex, U.K. CORTK[®] kits for corticosterone assay were obtained from Sorin, Gruppo Radiochimica, Saluggia, Italy. All other chemicals and reagents were of the purest grade available and purchased from Sigma, Poole, Dorset, U.K.

Animals and Treatment

Female Sprague-Dawley rats of body weight 250–350 g were used throughout. These animals were purchased from Olac, Bicester, Oxon., U.K., and maintained, six per cage, on Dixon's (Ware, Herts., U.K.) PRM diet. After seven days acclimatization to the standard laboratory conditions (temperature $21^{\circ} \pm 2^{\circ}\text{C}$, 60% relative humidity and 12-hour light-dark cycle), the experiments were conducted on naive (except where specifically mentioned) rats. The tests were begun between 9–11 a.m. after an overnight fast.

Animals, in groups of six, were anaesthetized by intraperitoneal administration of either hexobarbital (100 mg/kg), thiopentone (56 mg/kg), or quinalbarbitone/amylobarbitone (25 mg/kg). Michel[®] suture clips were inserted through each external pinna of the ears of each rat. Crocodile clips, leading from the terminals of NET stimulators, were attached to the suture clips on the ears of each rat individually. Treated animals were stimulated until they regained their righting reflex with a square wave current via these suture clip-electrodes at a constant voltage (1 V) and pulse

width (0.22 msec) which was monitored constantly on an oscilloscope (Advance Instruments, Hainault, Essex, U.K.) In the case of animals which received thiopentone or quinalbarbitone/amylobarbitone, only one frequency, 10 Hz, was applied. Groups of six animals, anaesthetized with hexobarbital, were electrically stimulated at one of the frequencies indicated in Table II. In the case of sham treated controls, the pinna of the rats were also connected to instruments, but these were not switched on.

Sleeping time was measured as the total time taken by the animals to regain their righting reflex. The return of the righting reflex was considered to be the point when the anaesthetized rat recovered sufficiently to rotate and place either opposite hind limb on the surface of the bench. Sham treated, and the group of animals in which electrostimulation exerted its maximal effect on sleeping time, were sacrificed by exsanguination via cardiac puncture. The serum was separated by centrifugation and stored at -70°C prior to analysis. The livers of these animals were also excised, homogenised in ice-cold 1.15% KCl, and the microsomes prepared by ultracentrifugation.

Prior treatment with naloxone was achieved by intraperitoneal administration of Narcan[®] (1 mg/kg) 1 hour before administration of hexobarbital (100 mg/kg) (Table 4).

In two further groups of six rats (one control and one sham), the effect of electrostimulation at 10 Hz on the hexobarbital-induced sleeping time was determined once daily for four successive days.

Assays

Microsomal protein and cytochrome P_{450} were determined by the familiar techniques previously described [Capel et al., 1979]. The aromatic hydroxylation of aniline and N-demethylation of aminopyrine were determined by the techniques cited by Kato and Gillette [1965]. Serum corticosterone was determined by a competitive protein binding radioimmunoassay [Fiorelli et al., 1972].

Statistical Analyses

The statistical significance between electrostimulated and sham treated rats was determined by unpaired Student's *t* test. The difference between means was considered significant when $p < 0.05$.

RESULTS

Female rats receiving electrostimulation regained their righting reflex more rapidly than sham-treated animals following administration of anaesthetic doses of either hexobarbital, thiopentone, or quinalbarbitone/amylobarbitone (Table 1). Two frequencies, either 10/Hz ($p < 0.001$) or 500 Hz ($p < 0.001$) were most effective in decreasing the hexobarbital-induced sleeping time (Table 2).

The treatment produced no significant difference in microsomal protein or cytochrome P_{450} levels (Table 3). Paradoxically, the two mixed function oxidase enzyme activities investigated, aminopyrine N-demethylase and aniline hydroxylase, were significantly lower ($p < 0.001$) in the electrostimulated animals than their sham treated littermates.

Pretreatment with naloxone increased the hexobarbital-induced sleeping times of the non-electrostimulated animals (Table 4). Electrostimulation at a frequency of 10 Hz was far less effective ($p < 0.001$) in reducing sleeping time following pretreatment with naloxone, whereas the reduction of sleeping time resulting from electrostimulation at 500 Hz was greatly enhanced ($p < 0.001$) by prior administration of this drug.

Administration of hexobarbital on successive days resulted in a decrease in sleeping time of the untreated animals. However, electrostimulation at 10 Hz reduced the sleeping time on each successive day by a similar percentage (Table 5). Rats receiving electrostimulation at 10 Hz had significantly lower ($p < 0.002$), and those stimulated at 500 Hz significantly higher ($p < 0.025$), serum cortisol levels than the sham treated controls (Table 6).

TABLE 1. The Effect of Electrostimulation at a Frequency of 10 Hz on the Sleeping Times Induced by Various Barbiturates in Female Rats

Barbiturate	Dose mg/kg i.p.	Sleeping time (min)		% Decrease in sleeping time
		stimulated	sham	
Hexobarbital	100	62 ± 10	84 ± 12	25
Pentothal	56	267 ± 33	371 ± 49	28
Tuinal	25	107 ± 10	123 ± 14	16

Results represent the mean ± S.D. of individual observations on six animals per group

TABLE 2. The Effect of Electrostimulation at Various Frequencies on the Hexobarbital (100 mg/kg)-Induced Sleeping Times in Female Rats

Frequency (Hz)	Sleeping time (min)	% Reduction in sleeping time
1	61 ± 12	23
5	56 ± 11	29
10	46 ± 8	42
15	59 ± 16	25
20	57 ± 6	28
30	54 ± 9	32
90	68 ± 6	14
100	72 ± 9	9
200	74 ± 13	5
500	48 ± 5	39
1000	58 ± 6	27
Sham (control)	79 ± 13	

Results represent the mean ± S.D. for individual assays on six animals in each group.

TABLE 3. The Effect of Electrostimulation (10 Hz) on Some Microsomal Enzyme Activities in Hexobarbital (100 mg/kg)-Anaesthetized Female Rats

Group	Body weight (g)	Microsomal protein (mg/g)	Cytochrome P ₄₅₀ ^a	Aniline hydroxylase ^b	Aminopyrine demethylase ^b
Electro- stimulated	303 ± 8	35.7 ± 3.8	0.86 ± 0.12	0.68 ± 0.04	0.08 ± 0.01
Sham	310 ± 10	32.8 ± 3.2	0.99 ± 0.21	0.86 ± 0.07	0.12 ± 0.01

Results represent the mean ± S.D. for duplicate assays on six individual rats in each group.

^aExpressed as nmole/mg protein.

^bExpressed as pg product formed/mg protein/min.

Electrostimulation on Sleeping Times

TABLE 4. The Effect of Prior Administration of Naloxone on the Decreased Hexobarbital Sleeping Times of Female Rats Induced by Electrostimulation at Frequencies of 10 or 500 Hz

Group	Naloxone ^a	Sleeping time (min)	% Decrease in sleeping time
Sham	+	108 ± 14	—
	-	87 ± 11	—
10 Hz	+	96 ± 8	12
	-	63 ± 7	28
500 Hz	-	37 ± 2	66
	-	71 ± 13	18

Results represent the mean ± S.D. for six animals in each group.

^a1 mg/kg administered one hour before hexobarbital.

TABLE 5. The Effect of Repeated Daily Electrostimulation (10 Hz) on the Hexobarbital-Induced Sleeping Times of Female Rats

Day	Group	Sleeping time (min)	% Decrease in sleeping time
2	control	84 ± 12	—
	electrostimulated	62 ± 15	26
3	control	75 ± 8	—
	electrostimulated	55 ± 11	27
4	control	73 ± 11	—
	electrostimulated	49 ± 8	33
5	control	76 ± 7	—
	electrostimulated	58 ± 9	24

Results represent the mean ± S.D. for six animals in each group.

TABLE 6. Serum Corticosterone Levels in Hexobarbital-Anaesthetized Female Rats Receiving Electrostimulation at Frequencies of Either 10 or 500 Hz

Group	Sleeping time (min)	% Decrease in sleeping time	Corticosterone level in plasma (ng/ml)
Sham	95 ± 18	—	91 ± 16
10 Hz	63 ± 2	34	48 ± 18
500 Hz	67 ± 13	29	130 ± 35

Results represent the mean ± S.D. for individual assays on six animals in each group.

DISCUSSION

The majority of the work previously conducted to elucidate the therapeutic applications of electrostimulation has concentrated on the demonstrable analgesic properties evoked by this form of treatment [Cheng and Pomeranz, 1979, 1980; Hiedl et al., 1979; Woolf, 1979]. The present experiment describes a model by which another effect, i.e. drug detoxication, may be investigated, and also indicates that the results obtained will vary according to the frequency (Hz) of the stimulation applied.

The observed decrease in sleeping times resulting from electrostimulation in the present experiment could be indicative of increased hepatic function. Paradoxically, for the substrates used, aniline and aminopyrine, electrostimulation inhibited these two mixed function oxidase activities. Male rats experience a large increase in hepatic microsomal mixed function oxidase activity after sexual maturation so that immature male rats are generally better indicators of the inducing effects of various xenobiotics [Conney, 1967]. Female rats were used in the present experiment, but the observed decrease in the *in vitro* microsomal enzyme activity indicates that the effect on sleeping time is not the result of stimulated hepatic enzyme activity.

Electrostimulation frequencies below 100 Hz have been termed "slow" and those above 100 Hz "fast" [Nathan, 1978]. Some contradictory reports have appeared regarding the "optimal frequency" to induce autoanalgesia [Cheng and Pomeranz, 1979; Hiedl et al., 1979; Nathan, 1978]. Most reports recommend slow frequencies [Cheng and Pomeranz, 1980; Hiedl et al., 1979], but comparisons are difficult because differing forms of electrostimulation at differing intensities have been employed. A recent article [Cheng and Pomeranz, 1979] describes two "efficacious frequencies" (4 and 200 Hz) for induction of autoanalgesia experimentally and suggests that these frequencies might elicit their response by differing mechanisms, implicating a serotonergic mechanism at fast-frequency stimulation [Cheng and Pomeranz, 1979]. The results of the present experiment indicate that some specific slow and fast electrostimulation frequencies enable barbiturate-anaesthetized rats to regain their righting reflex more rapidly but also suggest that differing biochemical mechanisms produce this response at the differing frequencies.

The drug naloxone, when administered at higher dose levels, is generally regarded as a specific opioid antagonist and, although it may have other pharmacological interactions in a system which might compromise the interpretation of its effects [Sawynok et al., 1979], its reversal of a particular action is at least presumptive evidence of the involvement of opioid peptides in that reaction [Cheng and Pomeranz, 1979; Sawynok et al., 1979]. It has been demonstrated that the analgesia produced by slow frequency electroacupuncture may be abolished by naloxone whereas fast frequency autoanalgesia is unaffected [Cheng and Pomeranz, 1979]. Similarly, the use of naloxone in the present experiment suggests that at the slow electrostimulation frequencies "opioid peptides" contribute to, or are responsible for, the decrease in sleeping time, whereas at fast frequencies they antagonise the effect.

It has been reported that the autoanalgesic effects induced by noxious stimuli, such as footshock or conditioned fear, cannot be completely inhibited by either naloxone or the ACTH inhibitor, dexamethasone [Chance and Rosecrans, 1979; Lewis et al., 1980]. Indeed, it has been reported that naltrexone pretreatment enhances footshock stress-induced analgesia [Amir and Amit, 1979]. If these observations on autoanalgesia may be compared with barbiturate narcosis, they add support to the hypothesis that fast frequency electrostimulation elicits its response by a differing biochemical mechanism from slow frequency electrostimulation and that the fast frequency probably evokes the systems which enable the animal to cope with noxious stimuli.

Further evidence that slow and fast frequency electrostimulation invoke differing biochemical mechanisms to decrease sleeping time is afforded by the observed difference in serum corticosterone levels. It is possible that fast frequency electrostimulation induces the biochemical systems associated with stressful stimuli. In the present study the microsomal enzyme activities were investigated only in animals which received slow frequency electrostimulation. The hormones induced by stressful stimuli, including cortisol, normally increase hepatic microsomal metabolism [Conney, 1967]. It is possible, therefore, that fast frequency electrostimulation might be decreasing sleeping

times by enhancing drug metabolism. Adrenocorticotrophic hormone and β -endorphin originate from the same neuropeptide precursor and it has been demonstrated that electroacupuncture increases production of both hormones [Cheng et al., 1979]. The endorphins produced by the fast frequency stimulation would ameliorate the stressful stimulus, which would inhibit any hepatostimulatory action of the stress-associated hormones.

Clearly, further work is necessary to establish the means by which both fast and slow frequency electrostimulation reduce barbiturate-induced sleeping time. The present experiment does, however, provide a model system by which differing forms of electrostimulation may be compared and also establishes that the frequency of the current influences the response. The apparent beneficial effects on detoxication and barbiturate narcosis of electrostimulation also indicates a possible basis for clinically observed efficacious actions in the treatment of patients with various forms of drug addiction [Gomez and Mikhail, 1979; Patterson, 1976].

REFERENCES

1. Amir, S. and Amit, Z.: Enhanced analgesic effect of stress after the administration of naltrexone in rats. *Eur. J. Pharmacol.* 59: 137-140, 1979.
2. Buckett, W.R.: Peripheral stimulation in mice induces short duration analgesia preventable by naloxone. *Eur. J. Pharmacol.* 58: 169-178, 1979.
3. Capel, I.D., Williams, D.C., Davey, R.W. and Patterson, M.A.: The amelioration of restraint stress by electrostimulation. *IRCS Med. Sci.* 7: 634, 1979.
4. Capel, I.D., Dorrell, H.M., Jenner, M., Pinnock, M.H. and Williams, D.C.: The effect of prolonged ethanol intake on some carcinogen-activating enzymes in mice. *Biochem. Pharmacol.* 28: 1139-1141, 1979.
5. Chance, W.T. and Rosecrans, J.A.: Lack of effect of naloxone on autoanalgesia. *Pharmacol. Biochem. Behav.* 11: 643-646, 1979.
6. Cheng, R., Pomeranz, B. and Yu, G.: Dexamethasone partially reduces and 2% saline-treatment abolishes electroacupuncture analgesia: These findings implicate pituitary endorphins. *Life Sci.* 24: 1481-1486, 1979.
7. Cheng, R.S.S. and Pomeranz, B.: Electroacupuncture analgesia could be mediated by at least two pain-relieving mechanisms, endorphin and non-endorphin systems. *Life Sci.* 25: 1957-1962, 1979.
8. Cheng, R.S.S. and Pomeranz, B.H.: Electroacupuncture analgesia is mediated by stereospecific opiate receptors and is reversed by antagonists of (type μ) receptors. *Life Sci.* 26: 631-638, 1980.
9. Conney, A.H.: Pharmacological implications of microsomal enzyme induction. *Pharmacol. Rev.* 19: 317-366, 1967.
10. Fiorelli, G., Piolanti, P., Forti, G., and Serio, M.: Determination of plasma corticosteroids and urinary cortisol by a competitive protein-binding method using dextran coated charcoal. *Clin. Chim. Acta.* 37: 179-187, 1972.
11. Gomez, E. and Mikhail, A.R.: Treatment of methadone withdrawal with electrotherapy (electrosleep). *Br. J. Psychiatr.* 134: 111-113, 1979.
12. Hiedl, P., Struppler, A. and Gessler, M.: Local analgesia by percutaneous electrical stimulation of sensory nerves. *Pain* 7: 129-134, 1979.
13. Karo, R. and Gillette, J.R.: Effect of starvation on NADPH dependent enzymes in liver microsomes of male and female rats. *J. Pharmacol. Exp. Ther.* 150: 279-284, 1965.
14. Lewis, J.W., Cannon, J.T. and Liebeskind, J.C.: Opioid and non-opioid mechanisms of stress analgesia. *Science* 208: 623-625, 1980.
15. Nathan, P.W.: Acupuncture analgesia. *TINS* 1(7): 21-23, 1978.
16. Patterson, M.A.: Effects of neuro-electric therapy (N.E.T.) in drug addiction: Interim report. Offprint from U.N. Bulletin on Narcotics, Vol. XXVII, 4: 55-62, 1976.
17. Sawynok, J., Pinsky, C. and LaBella, F.S.: On the specificity of naloxone as an opiate antagonist. *Life Sci.* 25: 1591-1600, 1979.
18. Woolf, C.J.: Transcutaneous electrical nerve stimulation and the reaction to experimental pain in human subjects. *Pain* 7: 115-127, 1979.