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CRANIAL IRRADIATION WITH GaAlAs LASER LEADS TO NALOXONE REVERSIBLE ANALGESIA IN RATS [1, 2]

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Summary.—Laser irradiation of the rat cranium can produce analgesia. The present experiment investigated the mechanism of such action. 27 rats received all possible combinations of laser (0, 6.4, and 12J/cm^2) and naloxone (0, 5, and 10 mg/kg) prior to a hot plate test. Laser (820 nm, KHz pulsing, Omega Laser Systems, London) was applied to the rats' skulls and hind paw lick latencies (in seconds) were recorded immediately, 30 min., and 24 hr. after the administration of treatment. When animals were tested immediately following laser irradiation at 12J/cm^2 significant analgesia resulted. Treatment with naloxone at either dose antagonised this effect, but naloxone produced no significant hyperalgesia when given alone. This suggests that opioid peptide mechanisms mediate the analgesic action of low-intensity laser irradiation of the cranium.

For the past twenty years low-intensity laser irradiation has been promoted as a safe and effective treatment for a multitude of neurological and musculoskeletal conditions (Basford, 1989). While the photobiostimulation of wound healing remains the primary usefulness of low-intensity laser treatment, many authors advocate its use for the relief of pain of various etiologies (Walker, 1983). Despite widespread positive findings in support of laser mediated analgesia, its mechanism of action remains unknown. Several clinical and laboratory-based studies suggest a possible neuropharmacological basis. The majority of such investigations indicate that laser irradiation of peripheral points, usually acupuncture points, can significantly alter the production and metabolism of a wide range of neurochemicals (Choi, Srikantha, & Whu, 1986; Vizi, Mester, Tisza, & Mester, 1977; Zarkovic, Manev, Pericic, Skala, Jurin, Persin, & Kubovic, 1989). However, it is generally agreed that there is a major central nervous system component in the perception of pain. A number of neurotransmitters have been implicated in this, including GABA, serotonin, and the endogenous opiates (Melzack & Wall, 1988). Recently it has been demonstrated that *in vitro* low-intensity laser irradiation of the central nervous system also produces significant neurochemical changes

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(Shen, Xiao, Lin, & Wang, 1982; Pikulev, Zynanova, Lavrova, Mostovnikov, & Khohlov, 1985; Zynanova, Lavrova, Pikulev, & Khripchenko, 1987; Lombard, Rossetti, Cassone, Urciuoli, & Rolfo, 1991). Further, it has been shown that *in vivo* irradiation of the rat brain produces statistically significant analgesia in the hot plate assay (Wedlock, Shephard, Allen, & Baxter, 1993; Wedlock, Shephard, Little, & McBurney, in press). The aim of the present study was to investigate the possible role of endogenous opiates in laser-mediated analgesia by assessing the interaction of naloxone with laser irradiation of the cranium.

Method

Twenty-seven adult male Sprague Dawley rats with an average weight of 350 gm were used. Animals were housed singly in solid bottomed cages at room temperature. Food and water were freely available.

Nociceptive responding was measured by the hot plate assay (Woolfe & MacDonald, 1944; Eddy & Leimbach, 1953) using a Harvard hot plate analgesia meter (Harvard Instruments, Ltd). Surface temperature was thermostatically controlled at 55 ± 1°C. Latencies of paw licks were recorded by an electronic timer and were defined as the time (in seconds) which elapsed between initial contact of the paw with the hot surface and raising the hind paw and turning the head to lick it. Animals were removed from the hot plate immediately after making this response. A cut-off point of 30 sec. was enforced.

Laser irradiation was delivered by a Biotherapy 3ml Gallium Aluminum Arsenide (GaAlAs; Omega Laser Systems, London). The parameters were Wavelength, 820 nm; Peak radiant power output, 1W; Average radiant power output, 100 mW; Average irradiance, 1W/cm^2; pulse repetition rate, 5KHz; Radiant exposures, 0, 6.4, and 12J/cm^2. During laser administration animals were restrained in the experimenter's hand while sham or laser treatment was applied to the shaved skull at a point immediately between the ears.

Naloxone Hydrochloride (Sigma Bio-chemicals, Ltd) was dissolved in 0.85% saline for intraperitoneal injection in a volume of 1 ml/kg. Saline acted as a control and saline or naloxone (5 or 10 mg/kg) injections were given 10 min. before exposure to the hot plate. Doses were calculated as salt.

Animals were thoroughly acclimatised to all aspects of the experimental procedure for three days and control lick latencies were recorded during this time. Animals then received all possible combinations of laser (sham, 6.4, and 12J/cm^2) and naloxone (0, 5, and 10 mg/kg) treatments in random order. Hind paw lick latencies were measured immediately, 30 min., and 24 hr. afterward. Therefore a period of 48 hr. elapsed between each laser and/or naloxone treatment. Three animals who quickly learnt to jump off the hot

plate were dropped from the experiment, leaving the 27 subjects detailed above.

Results were analyzed with two-way analysis of variance for repeated measures and planned comparisons between individual drug and/or laser conditions and appropriate controls.

Results

Results of the experiment are given in Table 1. The table and analysis below correspond to the data obtained immediately after laser irradiation. Results from retesting at 30 min. and 24 hr. produced no significant differences between conditions and are therefore not included in the analysis reported here.

TABLE 1
Means and Standard Errors of Lick Latencies After Different Doses of Naloxone and Laser Irradiation

Naloxone Dose (mg/kg)	Laser Dose (J/cm^2)					
	0		6.4		12	
	M	*SE*	*M*	*SE*	*M*	*SE*
0	9.1	0.6	9.1	0.6	11.4	0.8
5	9.4	0.6	7.8	0.8	7.5	0.5
10	8.1	0.7	8.3	0.5	7.6	0.6

Two-way analysis of variance showed a nonsignificant main effect for laser treatment ($F=0.70$), but a significant main effect of naloxone treatment ($F=7.24$, $p<.01$) and of the interaction between the two ($F=3.79$, $p<.01$).

Planned pairwise comparisons showed that in the absence of naloxone the $12J/cm^2$ dose of laser produced a statistically significant analgesic effect ($t=2.7$, $p<.01$). The addition of 5 and 10 mg/kg of naloxone significantly antagonised the hypoalgesic effect of $12J/cm^2$ laser irradiation ($t=4.62$, $p<.0001$; $t=4.38$, $p<.0001$, respectively). Neither dose of naloxone produced significant hyperalgesia in the absence of laser treatment.

Discussion

Results from this experiment confirm previous findings (Wedlock, *et al.*, 1993; Wedlock, *et al.*, in press) that *in vivo* low-intensity laser irradiation of the rat cranium can produce quantifiable analgesia in the hot plate assay. The hypoalgesia observed to date has been relatively weak and short in duration. However, findings from other clinical and laboratory studies (Ponnudurai, Zbuzek, & Wu, 1987; Lowe, Baxter, Walsh, & Allen, 1993) indicate that alterations in radiant exposures or pulse repetition rates may extend both the magnitude and duration of this effect.

Neither dose of naloxone produced a significant intrinsic hyperalgesic

action, yet both significantly attenuated laser analgesia. This selectivity of action does not reflect simply a "floor effect," since we have observed mean response latencies of about 5 sec. in other experiments. Rather, it implicates endogenous opiate systems in the mechanism of laser-induced analgesia.

Such naloxone reversible analgesia has also been demonstrated following electrical brain stimulation (Akil, Mayer, & Liebeskind, 1976), transcutaneous electrical nerve stimulation (Chapman & Benedetti, 1977), and acupuncture (Pomeranz & Chiu, 1976; Mayer, Price, & Rafii, 1977). These treatments are also thought to be opioid dependent. There is at present no direct evidence to suggest that low-intensity laser irradiation can penetrate skull bone. However, we suggest that in the present study such penetration and subsequent absorption of radiation by neural tissue may mediate the analgesia observed. There are two indirect lines of evidence that support this view. Firstly, the analgesia observed in this experiment and by Wedlock, *et al.* (in press) is of immediate onset and short duration (less than 30 minutes in the present study). Transcutaneous electrical nerve stimulation and electroacupuncture which are thought to activate endogenous opiate release via peripheral mechanisms tend to be slower in onset and much longer in duration (Melzack & Wall, 1988). The time course of the analgesia observed following low-intensity laser irradiation of the cranium is closely related to that of brain stimulation which is thought to activate directly endogenous opiate release from the mid-brain (Akil, *et al.*, 1976).

Secondly Shen, *et al.* (1982) have demonstrated alterations in neurotransmitter levels following low-intensity laser irradiation delivered directly to brain tissue via a fibre optic. Similar changes in neurotransmitter levels have been demonstrated following irradiation of the intact skull (Lombard, Rossetti, Cassone, Urciuoli, & Rolfo, 1991). Perhaps, then, opioid mechanisms underlie analgesia induced by several nonpharmacological modalities, including laser irradiation. Further research could determine whether laser irradiation of the cranium induces other behavioural effects associated with opioid peptide release.

REFERENCES

Akil, M., Mayer, D. J., & Liebeskind, J. C. (1976) Antagonism of stimulation produced analgesia by naloxone, a narcotic antagonist. *Science*, 191, 961-962.

Basford, J. R. (1989) Low energy laser therapy: controversies and new research findings. *Lasers in Surgery and Medicine*, 9, 1-5.

Chapman, R. C., & Benedetti, C. (1977) Analgesia following transcutaneous electrical nerve stimulation and its partial reversal by a narcotic antagonist. *Life Sciences*, 2, 1645-1648.

Choi, J. J., Srikantha, K., & Whu, W-H. (1986) A comparison of electroacupuncture, transcutaneous electrical nerve stimulation and laser photobiostimulation on pain relief and glucocorticoid secretion. *International Journal of Acupuncture and Electrotherapeutics Research*, 11, 45-51.

Eddy, N. B., & Leimbach, D. (1953) Synthetic analgesics: II. Dithienylbutenyl and pithienylbutylamines. *Journal of Pharmacology and Experimental Therapeutics*, 107, 385-393.

LOMBARD, A., ROSSETTI, V., CASSONE, M. C., URCIUOLI, R., & ROLFO, P. M. (1991) Neurotransmitter content and enzyme activity variations in rat brain following in vivo He-Ne laser irradiation. In S. Passarella, E. Quagliarello, & G. Jor (Eds.), *Round table on basic and applied research in photobiology and photomedicine*. Bari, Italy: Bayer. Pp. 6.

LOWE, A. S., BAXTER, G. D., WALSH, D. M., & ALLEN, J. M. (1993) The effect of low intensity laser irradiation upon conduction and skin temperature in the human median nerve. *Lasers in Surgery and Medicine*, 5, 8.

MAYER, D. J., PRICE, D. D., & RAFII, A. (1977) Antagonism of acupuncture analgesia in man by the narcotic antagonist naloxone. *Brain Research*, 121, 368-372.

MELZACK, R., & WALL, P. (1988) *The challenge of pain*. (2nd ed.) London: Penguin.

PIKULEV, A. T., ZYNANOVA, T. N., LAVROVA, V. M., MOSTOVNIKOV, V. A., & KHOHLOV, I. V. (1985) Effect of local laser irradiation on the activity of some enzymes of glutamic acid metabolism in rat tissue. *Radiobiologiia*, 35, 678-680.

POMERANZ, B., & CHIU, D. (1976) Naloxone blockade of acupuncture analgesia: endorphins implicated. *Life Sciences*, 19, 1757-1762.

PONNUDURAI, R. N., ZBUZEK, V. K., & WU, W. (1987) Hypoalgesic effect of laser photobiostimulation shown by rat tail flick test. *International Journal of Acupuncture and Electrotherapeutics Research*, 12, 93-100.

SHEN, Z., XIAO, J., LIN, S. Z., & WANG, L. H. (1982) Effects of low power laser beam guided by optic fibre on rat brain striatal mono-amines and amino acids. *Neuroscience Letters*, 32, 203-208.

VIZI, E. S., MESTER, E., TISZA, S., & MESTER, A. (1977) Acetylcholine effects of laser irradiation to Auerbach plexus in guinea pig ilium. *Journal of Neural Transmission*, 40, 305-308.

WALKER, J. (1983) Relief from chronic pain by low power laser irradiation. *Neuroscience Letters*, 43, 339-344.

WEDLOCK, P. M. (1995) Behavioural effects of low intensity laser irradiation of the rodent brain. Unpublished D.Phil. thesis, University of Ulster.

WEDLOCK, P. M., SHEPHARD, R. A., ALLEN, J. M., & BAXTER, G. D. (1993) Analgesia with cranial laser irradiation in rats is reversible with naloxone. *Irish Journal of Medical Science*, 162, 387.

WEDLOCK, P. M., SHEPHARD, R. A., LITTLE, C., MCBURNEY, F. (in press) Analgesic effects of cranial laser treatment in two rat nociception models. *Physiology and Behavior*.

WOOLFE, G., & MACDONALD, A. D. (1944) The evaluation of the analgesic action of pethidine hydrochloride (Demerol). *Journal of Pharmacology and Experimental Therapeutics*, 80, 300-307.

ZARKOVIC, N., MANEV, H., PERICIC, D., SKALA, K., JURIN, M., PERSIN, A., & KUBOVIC, M. (1989) Effect of semiconductor GaAs laser irradiation on pain perception in mice. *Lasers in Surgery and Medicine*, 9, 63-66.

ZYNANOVA, T. N., LAVROVA, V. M., PIKULEV, A. T., & KHRIPCHENKO, I. P. (1987) Catecholamine content and acetylcholinesterase activity in rat brain affected by laser radiation. *Radiobiologiia*, 227, 94-97.

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