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Research Paper

Central nor-adrenergic pathway: essential role in diffuse transcranial electrical stimulation- induced hypermotitity

S.O. Otimenyin*, U. A. Osunkwo
Department of Pharmacology, Faculty of Pharmaceutical Sciences, University of Jos
P.M.B. 2084, JOS, Plateau State, Nigeria
* Corresponding Author
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ABSTRACT

The involvement of nor-adrenergic pathway in diffuse transcranial electrical stimulation (DTES)-induced hypermotility was investigated, using a centrally acting nor-adrenergic pre-synaptic agonist. Wister rats were pretreated with clonidine (120 μg/Kg and 240 μg/Kg respectively), a pre-synaptic α_2 agonist. Clonidine pre-treated rats and control rats received DTES 30 minutes after treatment in a motility counting chamber. Administration of higher dose of Clonidine produced similar results with lower motility counts. Implying that clonidine reduced the sympathetic outflow to the periphery, and thus reduced rat's activity. These results suggest that DTES- induced hypermotility may have resulted from an increase in sympathetic outflow to the periphery, indicating that nor-adrenergic pathway is the essential mediator of DTES induced hypermotility.

Keywords: DTES; Central nor-adrenergic pathway; Nor-adrenergic receptors; hypermotility; Clonidine; Wistar rats.

INTRODUCTION

Electrical stimulation of the brain has been used as a method for stimulating the whole brain of an experimental animal. Diffuse transcranial electrical stimulation (DTES) delivers electric currents to the brain via ear clip electrodes. DTES evoke hypermotility in awake Wistar rats (Otimenyin, et al., 2004). It is similar to what is seen in man during electroshock (ECG), a method used in the management of depression in depressed patients.

Diffuse transcranial electrical stimulation of the brain of an animal has been suggested as a non-invasive method of stimulating the whole brain of the animal (Osunkwo, et al., 1994). Electrical stimulation of various centers in the brain causes emotional stress-like behavior (Bunag, et al. 1975; 1976). It mimics emotional stress, activates higher brain centers and is taught to increase sympathetic outflow to the periphery). Increased sympathetic outflow to the periphery results in hyper activity, which is observed as hypermotility and restlessness. In man, physiological response to emotional stress is known to induce transient "situational hypertension" and herperactivity in normal subjects (Var der Valk, 1957).

Electrical stimulation of specific brain areas (amygdala and locus coerulus) mimics' emotional stress in man, it induces cardio-acceleration and arterial hypertension (Stock, et al., 1961). To effect electrical stimulation of discrete brain regions requires rupture of the skin, skull and even brain tissue before inserting stimulating electrode. The process of rupturing the brain is painful and can at times destroy nearby brain cells and elicit unwanted responses.

Another method of inducing emotional stress-like behavior in the rats; is the pitting method (Grant, et al., 1985; Flayahan, et al., 1985). These methods are often useful, in inducing emotional stress like behaviours in experimental animals. Such animals are useful for the evaluation of central effects of promising drugs.

Some drugs (Morphine and related narcotics analgesics) stimulates motor activity (olivero, et al., 1974), an effect hypothesized to depend on brain dopamine (Carroll, et al., 1972; Di Chiara, et al., 1977). Recently, this hypothesis has been changed and alternative mechanisms (the involvement of nor-adrenaline) have been proposed (Vaccarino, et al., 1986). Otimenyin and Osunkwo, (2004) reported that diffuse transcranial electrical stimulation induces hyper motility in male and female wistar rats. They classified DTES as a non-invasive method of stimulating the central nervous system of an animal. Their work also showed that DTES- induced activity may be useful in the assay of anti-epileptic and anti-convulsants. What was not clear was the mechanism by which this effect is mediated.

To find out if nor-adrenergic pathway is involve in the induction of hypermotility by diffuse transcranial electrical stimulation, DTES, we have used clonidine a centrally acting presynaptic α_2 agonist. Clonidine has been reportedly used in the management of chronic post-traumatic stress disorder of war (Kolb, et al., 1984). The main use of clonidine is in the management of hypertension, (Rand, et al., 1994). It exerts its effects within the central nervous system, by stimulating pre-synaptic α_2 receptors in the brain stem, locus coeruleus, medullospinal nor-adrenergic pathway, medullary dorsal motor complex, intermediolacteral column, substantia gelatinosa and the dorsal horn of the spinal cord. Clonidine has also been used to significantly attenuate the stress response to laryngoscopy. Stimulation of pre-synaptic α_2 receptors by clonidine results in reduced sympathetic outflow from the central nervous system to the periphery. This might be the basis for its use in the management of stress related problems. Clonidine was used because of its selective effect on the nor-adrenergic pathways in the central nervous system.

The objective of this study was to establish the involvement of the brain sympathetic pathway in the induction of hypermotility by DTES, and to postulate this as the major mechanism by which DTES elicit it CNS effects- like the elevation of blood pressure (Osunkwo, et al., 1994).

MATERIALS AND METHODS

Adult male and female Wister rats (200-250g) inbred in the animal Production Unit of the National Veterinary Research Institute Vom were used for these studies. Animals had access to tap water and feed (24% protein: Pfizer Products, Lagos, Nigeria) *ad libitum.* Rats were housed at 25 ± 2^{o} C and acclimatized on approximately 12 hours light and 12 hours dark cycle in the animal house. They were randomly selected from colony of Male and Female rats. This experiment was approved by the ethical committee on the use of animals for laboratory research, University of Jos, in 1998. The experiments were conducted in accordance with the Guiding Principles in the Use of Animals in Toxicology, which were adopted by the Society of Toxicology in July, 1989, amended December, 2008.

Rats were divided into three groups of five rats each. Group one received distilled water intraperitoneally, while groups two and three received 120 and 240 µg/Kg of clonidine (Boehringer Ingelheim, Germany) respectively. After thirty minutes, DTES (5-10v, frequency 90Hz, pulse width 1 ms) was delivered to the groups via steel electrodes clipped on the left and right ear lobe of the rat. Motility counts were noted 10 seconds before, 10 seconds during, and 10 seconds after DTES administration (via a CFP stimulator model 8048, C. F. Palmer, London, UK.).

Stimulating voltage was determine after preliminary experiments were carried out to determine the suitable voltage for brain stimulation, 5v to 25v for 10 seconds produced reproducible actions and activity on rats. Five Volts and ten Volts respectively were then chosen for these experiments. Somatic and behavioral changes, including locomotor activity of wistar rats before, during and after electrical stimulation was noted.

Results are presented as means ± SEM and were analyzed using Student's t-test (one way), and *P* values less than 0.05 were considered significant (Snedecor, et al., 1967).

RESULTS

Diffuse transcranial electrical stimulation (DTES) caused a rise in the general activity of experimental animals. It was observed that motility count 10s before stimulation was 10.0±4.1. During stimulation, the motility count significantly increased to 81.0±13.5 and after stimulation it fell to 21.6 ±6.8. The value after stimulation was not significantly ($P>0.05$) different from the values before stimulation but was significantly different from the value ($P<0.001$) obtained during stimulation (Table 1).

Table- 1: Effect of DTES on Motility of conscious normal Wister rats.

Treatment of DTES	Motility counts/10 sec.	Behavioural effects
Before stimulation	10.0±4.1	Normal exploratory behavior
During stimulation	81.0 ±13.5	Pilo-errection, aggression, escapes attempts.
After stimulation	21.6 ±6.8	Calm,

- 10 animals in each group.
- Contact time = 10s; n=10.

In clonidine pretreated wistar rats, it was observed that Motility count before DTES were insignificantly higher than that of the untreated rats. Value obtained during stimulation of pretreated rats was significantly lower than values obtained during stimulation of control rats ($P < 0.05$) (Table 2).

Table- 2; Effect of DTES on Clonidine treated rats.

Treatment of DTES	Motility counts/10 sec.		Behavioral effects	
	(120 µg/Kg)	(240 µg/Kg)	(120 µg/Kg)	(240 µg/Kg)
Before stimulation	12.10±3.2	17.9±2.4	Normal exploratory behavior	Normal exploratory behavior
During stimulation	57.33 ± 7.4	41.0 ±6.8	Piloerrection, aggression, escape attempts	Piloerrection, aggression, escape attempts.
After stimulation	25.83±5.7	27.4 ±4.1	Calm, reduced exploratory behavior	Calm,

- 10 animals in each group.

It was also observed that after DTES the motility count significantly ($P<0.001$) reduced to 25.83±5.7 which was significantly ($P<0.01$) higher than the values before DTES and significantly ($P<0.001$) lower than the values during DTES (Table 2). Activity of rats was recorded by activity (motility) counter (Maurel-reny, et al., 1995).

The results obtained when the dose of clonidine was increased to 24 µg/100g was similar to the one obtained with lower dose except that the motility count during stimulation with 240 µg/Kg was lower than that of 120 microgram/Kg. (Table 2).

DISCUSSION

Low-amplitude DC electric fields are effective in suppressing epileptic activities in several *in vitro* epilepsy models. Depending on stimulation voltage, DC currents can produce either depression or excitation of the brain. Suppression (depression) thresholds are far below the voltage values required to produce excitation (Warren and Durand, 1998), of the brain. The inhibition mechanism is well established as membrane hyperpolarization (Durand and Bikson, 2001). Low voltage DC is important for the control of epilepsy. Higher DC voltage produces excitation of the brain (which manifests as hypermotility and over-activity in animals). Hypermotility observed mimics epilepsy like presentations. Stimulation of specific areas in the brain produced specific effects, which results from excitation of the region stimulated. Localized stimulation produce localized epilepsy, as seen in the stimulation of amygdala, (Nissinen, et al., 2000).

Electrical stimulation of rat's brain via ear clip electrodes produces an increase in locomotor activity (Otimenyin and Osunkwo, 2004). The increase in activity is likely due to the increase in the turnover of catecholamines in the brain, (Hollister, 1971), leading to an increase in the sympathetic outflow to the periphery. This may explain the hypermotility observed during sub-convulsive and convulsive diffuse transcranial electrical stimulation.

The mechanisms underlining diffuse transcranial electrical stimulation induced hypermotility in the animal may be the same as the mechanism involved in the increase in arterial blood pressure during electrical stimulation of the brain, which is thought to result from increase sympathetic vasomotor activity and adrenomedulary catecholamines (Morpugo, 1968; Haeusler, 1975). Von Eiff and Piekarski (1977) have postulated that stimuli creating defense and attack behavior, raises the blood pressure and muscle tone in animal and acts on the ``dynamogenic zone'' of the hypothalamus. It may therefore be conceived that diffuse transcranial electrical stimulation might result in the spread of electric current to the hypothalamus and the posterior nucleus to cause the hypermotility observed during DTES. The precise area of the brain activated during DTES to produce hypermotility is yet to be determined.

Results obtained from this study revealed that central noradrenergic pathway is involved in the mechanisms underlining hypermotility induced by DTES, confirming the earlier hypothesis (Morpugo, 1968; Haeusler, 1975). Clonidine acts on central presynaptic α_2 receptors that are largely localized in and around the tractus solitarius and the medulla, diminishing sympathetic outflow to the peripheri. Clonidine is the only presynaptic α_2 agonist available for human use. It is employed in the maintainance of perioperative cardiovascular and sympathoadrenal stability, sedation and analgesia (Kamibayashi, et al., 2000).

The pharmacology underlying the mechanism of action of α_2 agonists is well established (Scheinin, et al., 2000). The sedative action of presynaptic alpha$_2$-agonists has been linked to *locus coeruleus* (Correa-Sales, et al., 1992), which is the predominant nor-adrenergic nucleus in the brain stem. Mechanisms of the sedative effects of presynaptic α_2 agonists are attributed to changes in transmembrane ion conductance and hyperpolarization of excitable neural cells (Scheinin, et al., 2000), which makes nerve cells more difficult to excite to release neurotransmiter. This mechanism is different from the mechanism of action from general anaesthetics or

tranquilizers (e.g. benzodiazepines). Alpha$_2$ agonists have also been reported to have a significant hypnotic interaction with volatile anaesthetics (Aantaa, et al., 1997).

Clonidine exhibits analgesic properties, the mechanism and site of action of the sedative effect of the α_2 -agonists can be related to hyperpolarization of excitable cells in the *locus coeruleus*, the mechanism of action of the analgesic effect of alpha$_2$ agonists are more complex and yet to be fully investigated (Buerkle, et al., 1998).
Areas of the brain where clonidine exerts its action to attenuate DTES induced hypermotility is not clear, this study clearly shows that nor-adrenergic pathway is involved in DTES induced hypermotility. Further studies aimed at determining the specific brain sites of action is needed to further explain the mechanism of action underlining DTES induced hypermotility.

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