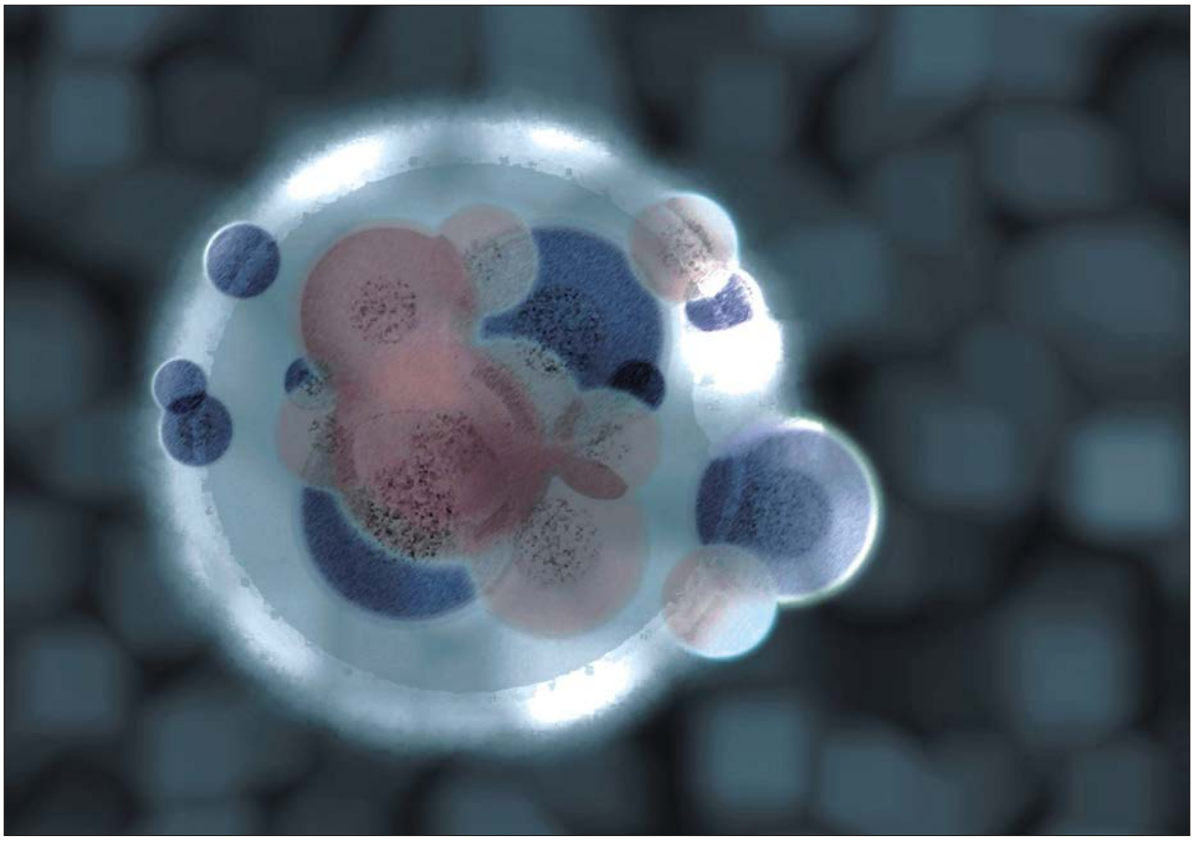

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The Involvement of Serotonin in Artemether-Induced Behavioural Activities

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Abstract: The aim of this study is to evaluate and confirm the possible neurotransmitter(s) involved in artemether-induced behavioural syndrome in rats. Rats were administered with 0.2 mL kg^{-1} normal saline and varying doses of i.m artemether (25, 50 and 100 mg kg^{-1}) for 6 consecutive days. Neurotoxic symptoms were observed with 50 and 100 mg kg^{-1} from day 6 upward. However, the behavioural syndrome was induced consistently at 100 mg kg^{-1} × 6 days specifically between day 8 and 9. Another group of rats were pre-treated with various drugs (apomorphine, morphine, naltrexone, diazepam, cyproheptadine and clozapine) 30 min prior i.m artemether for 6 consecutive days and the various symptoms were observed in each rat. Finally, rats exhibiting the behaviors were subjected to the characteristic 5-HT testing. At 50 mg kg^{-1} × 6 days, artemether induced mild symptoms. At 100 mg kg^{-1} × 6 days, artemether induced consistent behavioural symptoms. Pretreatment with various drugs did not suppress the syndrome at day 8, but cyproheptadine and clozapine suppressed the syndrome significantly at day 6. Behavioural testing also confirmed the characteristic 5-HT syndrome in rats exhibiting the symptoms. The result confirmed the involvement and possibly by an enhanced 5-HT neurotransmission in the mediation of the behavioural syndrome due to sub-chronic administration of i.m artemether in rats.

Key words: Artemether, behavioural activities, 5-HT (Serotonin) involvement

INTRODUCTION

Artemether is an oil-soluble derivative of artemisinin a sesquiterpene lactone endoperoxide with unusual peroxide linkages (Hien and White, 1993; Li and Wu, 1998; White and Olliaro, 1998). Artemisinin and its derivatives (arteether, artemether and artesunate) have been confirmed to be the best-tolerated and rapidly acting antimalarials known to date (White and Olliaro, 1998; Li and Wu, 1998). Recently, it has been reported that artemisinin and its derivatives given at a high parenteral doses causes selective brain stem neuropathy in laboratory animals (Dayan, 1998). The neurotoxic effects of these agents have been demonstrated in rats (Brewer *et al*., 1994; Kamchonwongpaisan *et al*., 1997), in male rhesus monkeys (Petras *et al.,* 1997).

Other neurological symptoms observed in experimental animals are; gait disturbances, loss of spinal and pain reflexes, restlessness, tremors, motor in-coordination, respiratory depression, diminished activity, convulsions and cardio-respiratory arrest (Hien and White, 1993). It was reported by the China Cooperative Research Group in 1982 that most of these symptoms become manifested at extremely high doses that is 5,000 fold higher than therapeutic doses in mice. Recent discovery of possible neurotoxic effects of these drugs in cases of acute overdose, cumulative doses arising from too frequent repeated use, abuse, misuse or when used prophylactically especially in countries where there is in -adequate control over drug use, call for more research work on the toxicity of the drug.

In virtually all the research works reported, the mechanism or neuro-transmitters involved in the mediation of the gross behaviours observed were not postulated or assessed. Hence, the aim of this study is to evaluate and confirm the specific neurotransmitter(s), or neuro-chemical pathways involved in artemether-induced stereotype behaviours in rats.

MATERIALS AND METHODS

Experimental animals: Swiss albino Wistar rats (150-250 g) were bred in the Department of Pharmacology Animal House, Obafemi Awolowo University, Ile-Ife, Nigeria. The rats were kept under standard laboratory conditions. They were fed on normal mouse cubes (Ladokun Feeds, Ibadan, Nigeria) and water *ad libitum*. All the rats were allowed to acclimatize to the laboratory

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environment for at least one week before commencement of experiment. The principle of laboratory animal care (NIH publication No. 85-23) guidelines and procedures were followed in this study (NIH publication revised, 1985).

Effects of different doses of i.m artemether: Twenty five rats were randomly allocated to 5 groups, each group comprised 5 rats each, the first group of 5 rats were administered with 0.2 mL kg^{-1} normal saline, while the remaining groups were administered with 25, 50 and 100 mg kg^{-1} i.m. artemether respectively daily for 6 consecutive days, according to the method of Dourish (1981). Each treated animal was observed singly inside plaxiglas's cage daily throughout the 6 days and up to 4 days after treatment. All the behavioural signs and symptoms were observed for 30 min and recorded. The weight loss for each treated group was also calculated at day 7 (24 h after last injection).

Effects of pretreatment with various agents prior 100 mg kg^{-1} artemether: Rats were randomly assigned to different groups of 5 rats each. Each group of rats were then pre-treated with 1 mg kg^{-1} apomorphine; 1 mg kg^{-1} morphine; 5 mg kg^{-1} naltexone; 10 mg kg^{-1} cyproheptadine p; 30 mg kg^{-1} clozapine; 2 mg kg^{-1} diazepam and 12.5 mg kg^{-1} chlorpromazine 30 min (i.p.) prior 100 mg kg^{-1} i.m. artemether; These agents were used to explore the involvement of dopaminergic, serotonergic, opiodergic, benzodiazepinergic and cholinergic neurotransmission in the mediation of behavioural effects of artemether (Dourish, 1981).

Behavioural testing of 5-HT syndrome: Rats treated with 100 mg kg^{-1} artemether were assessed for the characteristic 5-TH symptoms as described by Jacobs (1976) and Dourish (1981).

Drugs: Artemether-Paluther ® 80 mg mL^{-1} (Rhone-Poulec Rorer, France); Apomorphine HCl (sigma chemical USA); Clozapine (Sandoz); Morphine (Evans Med. Supplies Ltd.); Naltrexone (Evans med. Supplies); Chlorpromazine 50 mg L/2 mL (Health Pharm. Co. Ltd.); Inj. Valium-Diazepam 10 mg L/2 mL (Roche); Cyproheptadine (MSD).

Statistical analysis: The results were expressed as mean±SEM and were analysed using Kruska Wallis analysis of variance followed by Student t-test. Differences between the mean values were considered statistically significant at $p<0.05$.

RESULTS

All the rats in the control group did not exhibit any of the symptoms. At 25 mg kg^{-1} ×6 days, artemether induced only slight symptoms of hyperactivity, restlessness, circling, hypophagia and weight loss. At 50 mg kg^{-1} × 6 days, artemether induced moderate but inconsistent symptoms. At 100 mg kg^{-1} × 6 days, artemether consistently induced all the behavioural syndromes stated in Table 1. For the group treated with 100 mg kg^{-1} × 6 days, all the rats died before assessment day.

Effects of i.m artemether (100 mg kg^{-1} × 6 days) and of pretreatment with various agents on some artemether induced behavioural syndrome at day 6 and day 8, respectively. Clozapine and cyproheptadine successfully inhibited behavioral syndromes at days 6 but no effect on day 8. Clozapine and cyproheptadine suppressed the symptoms significantly, whereas, the other agents did not have any effect at day 6. But by day 8, all the rats in all the pretreatment groups exhibited similar symptoms similar to i.m 100 mg kg^{-1} artemether treated group only (Table 2 and 3).

Behavioural testing: General behavioral syndromes due to sub-chronic administration of 100 mg kg^{-1} day^{-1} of i.m artemether for 6 consecutive days showed the characteristic 5-HT behavioral syndromes (Jacobs, 1976; Dourish, 1981) of the six 5-HT behavioural syndromes of, resting tremor, rigidity or hypotonicity, hind limbs abduction, lateral head weaving and straub-tail, 5 of these were consistently observed (Table 4).

Confirmatory testing: For confirmation of 5-HT stimulation at least any 4 of the above components of the syndrome must be present during observation

Table 1: The behavioural syndromes of varying doses of i.m artemether for 6 consecutive days compared to control (0.2 mL mg kg^{-1} normal saline). Assessment was done between days 6 and 9

		I.M. Artemether mg kg^{-1} day^{-1} × 6 days		
Gross behavioural syndromes	0.2 mL kg^{-1} normal saline	25	50	100
Shaking	-	-	++	+++
Tremors	-	-	++	+++
Hyperactivity/Hyper-reactivity	-	+	++	+
Restlessness	-	+	++	+
Circling	-	-	++	+++
Convulsion	-	-	-	+++
Rigidity/Hypotonicity	-	-	-	+++
Hind limb abduction	-	-	-	+++
Lateral head weaving	-	-	-	+++
Reciprocal fore-paw padding	-	-	-	+++
Hypophagia	-	+	++	+++
Weight loss	-	+	++	+++

- = No sign observed, + = Slight symptoms observed, ++ = Moderate symptoms observed, +++ = Severe symptoms observed

Table 2: Symptoms observed due to i.m. artemether at varying doses and when pretreated with different drugs 30 min prior to 100 mg kg^{-1} day^{-1} 0.2 mL kg^{-1} artemether for 6 consecutive days. Assessment was carried out at day 6. Each animal was observed for 30 min. Only clozapine and cyproheptadine successfully inhibited shaking and tremor on day 6. However, apomorphine and diazepam did not cause any change in the symptoms

Treatment	Behaviours			
(× 6 consecutive days) n = 5	Shaking	Tremor	Convulsion	Circling
0.2 mL kg^{-1} i.m. Normal Saline	0	0	0	0
25 mg kg^{-1} i.m. artemether	0	0	0	0
50 mg kg^{-1} i.m. artemether	1	0	0	1
100 mg kg^{-1} i.m. artemether	2	2	2	2
0.5 mg kg^{-1} i.p apomorphine + 100 mg kg^{-1} i.m. artemether	2	2	0	0
10 mg kg^{-1} i.p Cyproheptadine + 100 mg kg^{-1} i.m. artemether	0	0	0	0
30 mg kg^{-1} i.p Clozapine + 100 mg kg^{-1} i.m. artemether	0	0	0	0
12.5 mg kg^{-1} i.p Chlorpromazine + 100 mg kg^{-1} i.m. artemether	1	1	0	0
2 mg kg^{-1} i.p Diazepam + 100 mg kg^{-1} i.m. artemether	2	2	0	0
1 mg kg^{-1} i.p Morphine + 100 mg kg^{-1} i.m. artemether	1	1	0	0
5 mg kg^{-1} Naltrexone + 100 mg kg^{-1} i.m. artemether	1	1	0	0

0 = not observed, 1 = mild (observed 1-4 times), 2 = moderate (observed 5-9 times)

Table 3: Symptoms observed due to i.m. artemether at varying doses and when pretreated with different drugs 30 min prior to 100 mg kg^{-1} day^{-1} artemether for 6 consecutive days. Assessment was done on Days 8 and 9. Each animal was observed for 30 min. By day 9, there is similarity in the severity of symptoms in all the pretreated groups and 100 mg kg^{-1} artemether treated group except that shaking and tremor were slightly less for clozapine, cyproheptadine and morphine pretreated groups

Treatments	Behaviours			
(x 6 consecutive days) n = 5	Shaking	Tremor	Convulsion	Circling
0.2 mL kg^{-1} i.m. Normal Saline	0	0	0	0
25 mg kg^{-1} i.m. artemether	0	0	0	0
50 mg kg^{-1} i.m. artemether	1	1	0	2
100 mg kg^{-1} i.m. artemether	3	3	3	3
0.5 mg kg^{-1} i.p apomorphine + 100 mg kg^{-1} i.m. artemether	3	3	3	3
10 mg kg^{-1} i.p Cyproheptadine + 100 mg kg^{-1} i.m. artemether	2	2	3	3
30 mg kg^{-1} i.p Clozapine + 100 mg kg^{-1} i.m. artemether	2	2	3	3
12.5 mg kg^{-1} i.p Chlorpromazine + 100 mg kg^{-1} i.m. artemether	3	3	3	3
2 mg kg^{-1} i.p Diazepam + 100 mg kg^{-1} i.m. artemether	3	3	3	3
1 mg kg^{-1} i.p Morphine + 100 mg kg^{-1} i.m. artemether	2	2	2	1
5 mg kg^{-1} Naltrexone + 100 mg kg^{-1} i.m. artemether	3	3	2	2

0 = not observed; 1 = mild (observed 1-4 times); 2 = moderate (observed 5-9 times); 3 = Severe (observed more than 10 times)

Table 4: Behavioural syndromes due to 100 mg kg^{-1} day^{-1} × 6 consecutive days of i.m. artemether in rats

5-HT behavioural syndromes	Comment
Resting Tremor	Present
Rigidity or hypotonicity	Present
Reciprocal fore paw padding	Present
Hind limbs abduction	Present
Lateral head weaving	Present
Straub tail	Not observed

(Jacobs, 1976; Dourish, 1981). From the Table 4, 5 of the components of the syndrome were observed, therefore, 5-HT stimulation or transmission is hereby implicated in the behavioral syndrome due to sub-chronic administration of artemether 100 mg kg^{-1} day^{-1} × 6 days in the rats.

DISCUSSION

When rats were administered artemether (100 mg kg^{-1} ×6 consecutive days), a number of behavioural syndromes became manifested (Table 1). These syndromes are; shaking, tremors, convulsion, circling, rigidity/hypotonicity, hind limbs abduction, lateral head weaving, reciprocal forepaw padding, hyperactivity or hyper-reactivity. From these syndromes, 2 major mechanisms could be proposed, the first one is the involvement of 5-HT transmission and the associated symptoms include, Lateral head weaving, tremors, hind limbs abduction, reciprocal forepaw padding/treading, hypophagia and rigidity/hypotonicity (Jacobs, 1976; Dourish, 1981). The second major mechanism involves the dopaminergic neuro-transmission and associated symptoms include, shaking, circling and hyper-activity (Dickinson and Curson, 1983; Carter and Pycock, 1978; Costal *et al*., 1979, 1983; Lees *et al*., 1979; Volkman *et al*., 1978; Roffman *et al*., 1978; Jones *et al*., 1981). The other mechanisms that can be proposed is the inhibition of GABA neuro-transmission causing convulsion.

From the results of behavioural testing in Table 4 and 5 of the behavioural syndromes of 5-HT were observed, thus it could be proposed that 5-HT neuro transmission may be the major pathway through which artemether-induced behaviours are mediated in rats. Thus, resting tremor, rigidity, reciprocal forepaw padding, hind limbs abduction and lateral head weaving are used to

confirm the involvement of 5-HT neuro-transmission in such behavioural syndromes (Jacobs, 1976; Dourish, 1981). The results also showed that artemether-induced highly significant anorexia and weight loss in laboratory rats in a dose-dependant pattern (Table 1).

From the results obtained in the study, it could be suggested that the major mechanism by which artemether induced behavioural syndromes in laboratory rats could be enhanced 5-HT (Serotonin) neuro-transmission.

CONCLUSIONS

Increased 5-HT Neuro-transmission is hereby proposed as the main mechanism by which artemether induced behavioural syndromes in rats.

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