

## ALDRIN-INDUCED LOCOMOTOR ACTIVITY: POSSIBLE INVOLVEMENT OF THE CENTRAL GABAERGIC-CHOLINERGIC-DOPAMINERGIC INTERACTION

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*Aldrin-induced locomotor activity: possible involvement of the central GABAergic-cholinergic-dopaminergic interaction. Sk. JAMALUDDIN, M.K. PODDAR. Pol. J. Pharmacol., 2001, 53, 21–30.*

Aldrin (5 mg/kg/day, *po*) under nontolerant condition, administered either for a single day or for 12 consecutive days, enhanced locomotor activity (LA) of rats. The increase in LA was greater in rats treated with aldrin for 12 consecutive days than that observed with a single dose. The aim of the present study is to evaluate the involvement of possible interactions of central GABAergic, cholinergic and dopaminergic systems using their agonist(s) and antagonist(s) in the regulation of LA in aldrin nontolerant rats. Administration of either L-DOPA along with carbidopa or bicuculline potentiated aldrin-induced increase in LA under nontolerant condition as well as LA of the control rats. Treatment with muscimol, haloperidol, atropine or physostigmine all decreased the LA of both aldrin nontolerant and control rats. Further, the application of (a) haloperidol along with bicuculline, atropine or physostigmine and (b) physostigmine along with bicuculline or L-DOPA + carbidopa significantly reduced LA but L-DOPA + carbidopa along with atropine or bicuculline increased LA of the control rats. These agonist(s)/antagonist(s)-induced decrease or increase in LA of the control rats were attenuated or potentiated, respectively, when those agonist(s)/antagonist(s) under abovementioned condition were administered to aldrin nontolerant rats. The attenuating or potentiating effects of aldrin on agonist(s)/antagonist(s) (either individually or in different combinations)-induced change in LA were greater in rats treated with aldrin for 12 consecutive days than that observed with a single-dose aldrin treatment. These results suggest that aldrin, under nontolerant condition, reduces central GABAergic activity and increases LA by activating dopaminergic system *via* inhibition of cholinergic activity. The treatment with aldrin for 12 consecutive days produces greater effect than that caused by a single-day treatment.

**Key words:** *aldrin, locomotor activity, nontolerant, GABA, choline, dopamine*

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*Abbreviations:* CNS – central nervous system, DA – dopamine, L-DOPA – L-3,4-dihydroxyphenylalanine, GABA –  $\gamma$ -aminobutyric acid, LA – locomotor activity

## INTRODUCTION

Aldrin belonging to a cyclodiene group of pesticides, has long been used to control various pests [31]. The cyclodienes have a potent excitatory action on the nervous system [3, 17] and cause reflex excitability, convulsions, bradycardia and vasodilation [9]. The predominant signs of aldrin poisoning are motor incoordination, tremors, muscular weakness and nervousness [11, 27]. Like other cyclodienes, aldrin acts as an antagonist of  $\gamma$ -aminobutyric acid (GABA) ionotropic receptor [7, 25]. Bloomquist and Soderlund [3] have also stated that aldrin is a potent and effective inhibitor of GABA-gated chloride uptake, which results in only partial repolarization of the neurons. This leads to a state of the central nervous system (CNS) excitation [14]. The CNS excitation caused by aldrin has been found to be expressed in the form of an increase in locomotor activity (LA) of animals [1]. LA may be measured by counting vertical rearing frequency [12] and it is known that vertical rearing behavior is more related to vertical exploration in addition to locomotion [13]. It is also believed that exploratory behavior might be linked to an internal drive state. Exploration has been viewed in the context of acquisition of information(s). This cognitive framework coordinates the interaction of the organism with the environment and the processes associated with information gathering [13]. In this connection, it is important to note that aldrin at lower dose (5 mg/kg, *po*) evoked increase in LA [1]; whereas higher dose of aldrin (60 mg/kg, *po*) induced seizures [17]. Recently, we have shown that the inhibition of the central GABAergic activity induced by short-term aldrin treatment may be a cause of stimulation of LA in aldrin nontolerant rats. Aldrin nontolerant rats are those which exhibited aldrin-induced significant stimulation of LA as described elsewhere [our unpublished data]. Ladinsky et al. [16] have shown that the GABA antagonist, picrotoxin, increases the utilization of striatal acetylcholine by disinhibition of the nigrostriatal dopaminergic interneuron. GABA is also known to interact with dopamine (DA) and acetylcholine and also to interregulate their activities in the control of diffe-

rent behaviors [2, 4, 18, 21, 23, 26]. In the present study, the authors attempt to evaluate the involvement of possible interactions of central GABAergic, cholinergic and dopaminergic systems using their agonist(s) and antagonist(s), in the regulation of LA in aldrin nontolerant rats.

## MATERIALS and METHODS

### Animals

Adult male albino rats (120–130 g) of Charles Foster strain, were kept under 12 h light/12 h dark cycle at room temperature ( $28 \pm 0.5^\circ\text{C}$ ) with constant relative humidity ( $85 \pm 5\%$ ) and were maintained on a standard laboratory diet with water available *ad libitum*. LA of the rats was measured between 10.00 a.m. – 12.00 (noon) throughout the experiments.

### Chemicals

Muscimol, bicuculline, physostigmine, atropine sulphate and L-DOPA were purchased from Sigma Chemical Company (St. Louis, MO, USA). Carbidopa (peripheral decarboxylase inhibitor) was purchased from Indian Drug Pharmaceuticals (Hyderabad, India) and aldrin was obtained as a gift from ECI Agrochem (Pvt) Ltd. (Calcutta, India). Haloperidol was purchased from Searle Ltd. (Mumbai, India). All other reagents used in the present study were of analytical grade.

Physostigmine, muscimol, haloperidol and atropine sulphate were solubilized in distilled water. Bicuculline was dissolved in 0.01 M HCl. Aldrin was dissolved in groundnut oil, and L-DOPA and carbidopa were suspended in distilled water (immediately before use) and were given orally (*po*). All other drugs were administered intraperitoneally (*ip*).

### Treatment of animals with agonist(s) and antagonist(s) of different neurotransmitters

Rats were divided into eight different groups. Each group was divided into different subgroups. Each group contained 6–8 animals. Rats of each subgroup of group 1 were treated with agonist or antagonist of different neurotransmitters used in the present study either individually or in different combination(s). Subgroups of group 2 of rats were treated with vehicle of agonist(s) or antagonist(s) of different neurotransmitters (as used in the sub-

groups of group 1) either individually or in different combination(s) and were considered as control of the corresponding experimental subgroups of group 1. Rats of each subgroup of group 3 pre-treated with single dose of aldrin (5 mg/kg, *po*) were treated with agonist or antagonist either individually or in different combination(s). Rats of subgroups of group 4 were treated with vehicle (groundnut oil) of aldrin followed by treatment with agonist(s) or antagonist(s) (as used in the subgroups of group 1) either individually or in different combination(s). Subgroups of fifth group of rats were treated with vehicle of aldrin and vehicle of agonist(s) or antagonist(s) of different neurotransmitters (as used in the subgroups of group 1) either individually or in different combination(s) and these subgroups were considered as the control of the corresponding experimental subgroups of group 3. Rats of each subgroup of group 6 pre-treated with aldrin (5 mg/kg/day, *po*) for 12 consecutive days were treated with agonist(s) or antagonist(s) of different neurotransmitters either individually or in different combination(s) on the last day of aldrin treatment. Rats of subgroups of group 7 were treated with vehicle of aldrin for 12 consecutive days and they were also treated with agonist(s) or antagonist(s) of different neurotransmitters (as used in subgroups of group 1) either individually or in different combination(s) on the last day of vehicle of aldrin treatment. The vehicle of aldrin was administered to rats of each subgroup of group 8 for 12 consecutive days followed by administration of the vehicle of agonist(s) or antagonist(s) of different neurotransmitters (as used in the subgroups of group 1) either individually or in different combination(s) on the last day of the vehicle of aldrin treatment and these subgroups were considered as the control of the corresponding experimental subgroups of group 6.

Different combination of the agonist(s) and antagonist(s) of neurotransmitters used in the present study under various conditions were administered in such a way so that the time of their peak action coincide with the time of measurement of LA (as stated in detail in Tables 1–4). The corresponding control animals were also maintained under comparable time lag following the administration of respective vehicle. The doses, volume, routes of administration and time of exposure to aldrin and different agonist(s)/antagonist(s) and/or their corre-

sponding vehicle used in rats of different groups are described in detail in legends of Tables 1–4.

### Behavioral ratings of rat locomotor activity

The vertical rearing frequency of each animal was measured (between 10.00 a.m. – 12.00 noon) during 5 min observation period to monitor LA following the method of Keenan and Johnson [12] as described by Mukhopadhyay et al. [22]. The animals treated either with agonist(s) and antagonist(s) of GABAergic, cholinergic and dopaminergic receptor or their corresponding vehicles (according to the condition cited in the tables) were gently placed back in their home cages for a period depending on the agonist(s)/antagonist(s) administered and then they were transferred to a transparent plastic chamber (24 × 24 × 20 cm) illuminated with an electrical lamp at the top. The vertical rearing frequency was measured by an electrical device based on the capacitance change proportional to the distance between the animals' head and the probe.

### Statistical analysis

The statistical significance of differences between mean values was assessed by ANOVA (analysis of variance) using Tukey test [30] unless otherwise mentioned.

## RESULTS

Administration of a single dose of aldrin (5 mg/kg, *po*) significantly increased (68%;  $F = 38.81$ ,  $p < 0.001$ ) the LA of rats (Tab. 1). Treatment with aldrin (5 mg/kg/day, *po*) for 12 consecutive days also significantly increased (112%;  $F = 53.12$ ,  $p < 0.001$ ) the LA of rats (Tab. 3) with respect to control and with respect to that observed with a single dose of aldrin (Tab. 3) (26%;  $F = 90.64$ ,  $p < 0.001$ ). Muscimol, a GABA receptor agonist (1 mg/kg, *ip*) (76%;  $F = 47.80$ ,  $p < 0.001$ ), or dopaminergic receptor antagonist, haloperidol (1 mg/kg, *ip*) (82%;  $F = 45.14$ ,  $p < 0.001$ ), or cholinergic receptor agonist, physostigmine (0.2 mg/kg, *ip*) (82%;  $F = 53.65$ ,  $p < 0.001$ ), significantly reduced the LA with respect to their corresponding control (Tab. 1). The muscimol-, haloperidol- or physostigmine-induced reduction in LA was attenuated when muscimol (215%;  $F = 152.60$ ,  $p < 0.001$ ) or haloperidol (250%;  $F = 142.89$ ,  $p < 0.001$ ) or physostigmine (265%;  $F = 136.86$ ,  $p < 0.001$ ) were individually administered to aldrin (with a single

dose) treated rats (Tab. 1). These attenuating effects of aldrin on muscimol-, haloperidol- or physostigmine-induced reduction of LA was greater when rats were treated with aldrin for 12 consecutive days (Tab. 3). On the other hand, bicuculline (1 mg/kg, *ip*) (96%;  $F = 49.58$ ,  $p < 0.001$ ), a GABA receptor antagonist, or dopaminergic receptor agonist, L-DOPA (100 mg/kg, *po*) + carbidopa (10 mg/kg, *po*) (42%;  $F = 33.62$ ,  $p < 0.001$ ), significantly increased the LA in comparison with the corresponding control (Tab. 1). The increase in LA after bicuculline or L-DOPA + carbidopa was potentiated when bicuculline (19%;  $F = 35.82$ ,  $p < 0.001$ ) or L-DOPA + carbidopa (33%;  $F = 47.52$ ,  $p < 0.001$ ) were individually administered to aldrin (at a single dose)-treated rats (Tab. 1). These potentiating effects of aldrin on bicuculline- or L-DOPA + carbidopa-induced increase in LA was greater when rats were treated with aldrin for 12 consecutive days (Tab. 3). A non-significant reduction in LA was observed with cholinergic receptor antagonist, atropine (5 mg/kg, *ip*) in comparison with the corresponding control (Tab. 1). Further, it was noted that aldrin (single dose or 12 consecutive

days of treatment)-induced increase in LA was significantly reduced with muscimol, haloperidol, physostigmine or atropine but bicuculline or L-DOPA + carbidopa significantly potentiated the aldrin-induced LA (Tables 1 and 3) under similar conditions.

Haloperidol along with bicuculline (69%;  $F = 112.78$ ,  $p < 0.001$ ), atropine (100%) or physostigmine (100%), bicuculline along with physostigmine (56%;  $F = 89.65$ ,  $p < 0.001$ ) and L-DOPA + carbidopa along with physostigmine (52%;  $F = 92.38$ ,  $p < 0.001$ ) all significantly reduced LA in comparison with the corresponding control (Tab. 2). Reduction of LA induced by the abovementioned combination(s) of agonist(s)/antagonist(s) was attenuated when haloperidol along with bicuculline (67%;  $F = 110.60$ ,  $p < 0.001$ ), atropine or physostigmine, bicuculline along with physostigmine (184%;  $F = 163.84$ ,  $p < 0.001$ ) and L-DOPA + carbidopa along with physostigmine (150%;  $F = 141.58$ ,  $p < 0.001$ ) were administered to aldrin (at a single dose) treated rats (Tab. 2). These attenuating effects of aldrin on reduction of LA induced by the abovementioned combination(s) of agonist(s)/antagonist(s)

Table 1. Effect of single administration of agonist(s) and antagonist(s) of GABAergic, cholinergic and dopaminergic neurons on locomotor activity induced by single dose of aldrin in adult male rats

Treatment with agonist(s)/antagonist(s)	Locomotor activity		
	Vehicle of aldrin ± vehicle of agonist(s)/antagonist(s)*	Vehicle of aldrin ± agonist(s)/antagonist(s)**	Aldrin (5 mg/kg, <i>po</i> ) ± agonist(s)/antagonist(s)
Control	22.65 ± 1.72	22.65 ± 1.72	37.98 ± 1.76 <sup>a</sup>
Muscimol (1 mg/kg, <i>ip</i> )	23.62 ± 1.85	5.64 ± 0.50 <sup>a</sup>	17.76 ± 1.25 <sup>a</sup>
Bicuculline (1 mg/kg, <i>ip</i> )	23.49 ± 1.66	46.05 ± 2.99 <sup>a</sup>	54.59 ± 2.17 <sup>a</sup>
L-DOPA (100 mg/kg, <i>po</i> ) + carbidopa (10 mg/kg, <i>po</i> )	24.07 ± 1.86	34.40 ± 2.11 <sup>a</sup>	45.64 ± 2.48 <sup>a</sup>
Haloperidol (1 mg/kg, <i>ip</i> )	23.80 ± 1.75	4.33 ± 0.45 <sup>a</sup>	15.15 ± 1.04 <sup>a</sup>
Atropine (5 mg/kg, <i>ip</i> )	23.94 ± 1.85	18.75 ± 2.00	28.11 ± 1.76 <sup>a</sup>
Physostigmine (0.2 mg/kg, <i>ip</i> )	23.62 ± 1.76	4.14 ± 0.37 <sup>a</sup>	15.08 ± 1.36 <sup>a</sup>

Results are expressed as means ± SEM of 6–8 separate observations. Locomotor activity of control rats (without any vehicle treatment) was 22.54 ± 1.71. Bicuculline, muscimol, haloperidol, atropine or their corresponding vehicles were injected 30 min, L-DOPA + carbidopa or its corresponding vehicle 60 min and physostigmine or its vehicle 15 min prior to measurement of LA. LA was measured 2 h after aldrin or its vehicle administration. The total volume of agonist(s)/antagonist(s) including aldrin either individually or in different combinations was 0.2 ml. Rats treated with groundnut oil (vehicle of aldrin), 0.01 M HCl (vehicle of bicuculline), water (vehicle of L-DOPA + carbidopa) and saline (vehicle of muscimol, haloperidol, atropine and physostigmine) were considered as the control of the corresponding experimental groups. \* No significant difference between the LA of rats treated with the vehicle of aldrin along with the vehicle of agonist(s)/antagonist(s) of different neurotransmitters and the LA of rats treated with the vehicle of agonist(s)/antagonist(s) of different neurotransmitters (results not shown). \*\* No significant difference between the LA of rats treated with the vehicle of aldrin along with the agonist(s)/antagonist(s) of different neurotransmitters and the LA of rats treated with the agonist(s)/antagonist(s) of different neurotransmitters (results not shown). Dosages and routes of administration of different agonist(s)/antagonist(s) are presented in the parenthesis. Significantly different <sup>a</sup>  $p < 0.001$  using Tukey test for ANOVA

Table 2. Effect of single administration of different combination(s) of agonist(s) and antagonist(s) of GABAergic, cholinergic and dopaminergic neurons on locomotor activity induced by a single dose of aldrin in adult male rats

Treatment with agonist(s)/antagonist(s)	Locomotor activity		
	Vehicle of aldrin ± vehicle of agonist(s)/antagonist(s)*	Vehicle of aldrin ± agonist(s)/antagonist(s)**	Aldrin (5 mg/kg, <i>po</i> ) ± agonist(s)/antagonist(s)
Control	22.65 ± 1.72	22.65 ± 1.72	37.98 ± 1.76 <sup>a</sup>
Bicuculline + atropine	23.55 ± 1.76	24.93 ± 1.87	36.13 ± 1.44 <sup>a</sup>
Bicuculline + physostigmine	23.73 ± 1.80	10.55 ± 0.72 <sup>a</sup>	29.87 ± 1.78 <sup>a</sup>
L-DOPA + carbidopa + atropine	23.94 ± 1.62	29.75 ± 1.89 <sup>a</sup>	36.00 ± 1.86 <sup>a</sup>
L-DOPA + carbidopa + bicuculline	24.48 ± 1.84	54.10 ± 2.92 <sup>a</sup>	65.29 ± 4.11 <sup>a</sup>
Haloperidol + bicuculline	23.17 ± 1.55	7.26 ± 0.50 <sup>a</sup>	12.13 ± 1.09 <sup>a</sup>
Haloperidol + atropine	23.35 ± 1.71	0.0 <sup>a</sup>	23.51 ± 1.35 <sup>a</sup>
Haloperidol + physostigmine	23.94 ± 1.62	0.0 <sup>a</sup>	12.22 ± 0.95 <sup>a</sup>
L-DOPA + carbidopa + physostigmine	23.55 ± 1.76	11.25 ± 1.01 <sup>a</sup>	28.11 ± 2.30 <sup>a</sup>
L-DOPA + carbidopa + atropine + bicuculline	24.07 ± 1.62	42.44 ± 1.87 <sup>a</sup>	46.93 ± 3.20 <sup>a</sup>
L-DOPA + carbidopa + physostigmine + bicuculline	23.76 ± 1.54	39.83 ± 1.90 <sup>a</sup>	45.31 ± 3.40 <sup>a</sup>

Results are expressed as means ± SEM of 6–8 separate observations. Locomotor activity of control rats (without any vehicle treatment) was 22.54 ± 1.71. \* No significant difference between the LA of rats treated with the vehicle of aldrin along with the vehicle of agonist(s)/antagonist(s) of different neurotransmitters and the LA of rats treated with the vehicle of agonist(s)/antagonist(s) of different neurotransmitters (results not shown). \*\* No significant difference between the LA of rats treated with the vehicle of aldrin along with the agonist(s)/antagonist(s) of different neurotransmitters and the LA of rats treated with the agonist(s)/antagonist(s) of different neurotransmitters (results not shown). Other details are the same as described in the legend of Table 1. Significantly different <sup>a</sup>  $p < 0.001$  using Tukey test for ANOVA

were greater when rats were treated with aldrin for 12 consecutive days (Tab. 4). L-DOPA + carbidopa along with atropine (24%;  $F = 38.42$ ,  $p < 0.001$ ) or bicuculline (121%;  $F = 132.76$ ,  $p < 0.001$ ) significantly increased the LA in comparison with the corresponding control (Tab. 2). The increase in LA evoked by the above combination(s) of agonist(s)/antagonist(s) was potentiated when L-DOPA + carbidopa along with atropine (21%;  $F = 33.46$ ,  $p < 0.001$ ) or bicuculline (21%;  $F = 36.82$ ,  $p < 0.001$ ) were administered to aldrin (at a single dose) treated rats (Tab. 2). These potentiating effects of aldrin on induction of LA by L-DOPA + carbidopa along with atropine or bicuculline was greater when rats were treated with aldrin for 12 consecutive days (Tab. 4). The administration of bicuculline along with atropine did not affect the control and aldrin treated rats (both after single and 12-day treatment). Further, it was noted that aldrin (single or 12-day treatment)-induced increase in LA was significantly attenuated by bicuculline in the presence of physostigmine, haloperidol in the presence of atropine and L-DOPA + carbidopa

in the presence of physostigmine, but L-DOPA + carbidopa along with bicuculline significantly potentiated the aldrin-induced LA under similar conditions (Tabs. 2 and 4). L-DOPA + carbidopa along with bicuculline and atropine (76%;  $F = 92.86$ ,  $p < 0.001$ ) or physostigmine (69%;  $F = 72.46$ ,  $p < 0.001$ ) significantly increased LA in comparison with the corresponding control. The increase in LA evoked by the treatment with L-DOPA + carbidopa along with bicuculline and atropine or physostigmine were potentiated when they were administered to aldrin (at a single dose)-treated rats (Tab. 2). This potentiating effects of aldrin on induction of LA by L-DOPA + carbidopa along with bicuculline and atropine or physostigmine was further potentiated when rats were treated with aldrin for 12 consecutive days (Tab. 4).

Further, it was noted that bicuculline-induced increase in LA in the presence of aldrin (Tab. 1) was significantly reduced by atropine (34%;  $F = 96.85$ ,  $p < 0.001$ ), physostigmine (45%;  $F = 88.62$ ,  $p < 0.001$ ) or haloperidol (78%;  $F = 112.72$ ,  $p < 0.001$ ) (Tab. 2). The L-DOPA + carbidopa-induced

Table 3. Effect of single administration of agonist(s) and antagonist(s) of GABAergic, cholinergic and dopaminergic neurons on locomotor activity induced by aldrin treatment for 12 consecutive days in adult male rats

Treatment with agonist(s)/antagonist(s)	Locomotor activity		
	Vehicle of aldrin ± vehicle of agonist(s)/antagonist(s)*	Vehicle of aldrin ± agonist(s)/antagonist(s)**	Aldrin (5 mg/kg/day, po) ± agonist(s)/antagonist(s)
Control	22.56 ± 1.65	22.56 ± 1.65	47.78 ± 3.00 <sup>a</sup>
Muscimol	23.35 ± 1.64	5.30 ± 0.51 <sup>a</sup>	24.75 ± 1.52 <sup>a</sup>
Bicuculline	23.85 ± 1.80	45.60 ± 3.40 <sup>a</sup>	76.07 ± 3.80 <sup>a</sup>
L-DOPA + carbidopa	23.49 ± 1.62	33.99 ± 1.73 <sup>a</sup>	50.83 ± 1.84 <sup>a</sup>
Haloperidol	23.49 ± 1.62	4.15 ± 0.48 <sup>a</sup>	22.27 ± 0.82 <sup>a</sup>
Atropine	23.67 ± 1.53	17.92 ± 1.92	37.19 ± 1.42 <sup>a</sup>
Physostigmine	23.31 ± 1.68	4.06 ± 0.39 <sup>a</sup>	21.82 ± 1.03 <sup>a</sup>

Results are expressed as means ± SEM of 6–8 separate observations. Locomotor activity of control rats (without any vehicle treatment) was 22.54 ± 1.71. The agonist(s)/antagonist(s) were administered on the last day of aldrin treatment. \* No significant difference between the LA of rats treated with the vehicle of aldrin for 12 consecutive days along with the vehicle of agonist(s)/antagonist(s) of different neurotransmitters on the last day of vehicle of aldrin treatment and the LA of rats treated with the vehicle of agonist(s)/antagonist(s) of different neurotransmitters (results not shown). \*\* No significant difference between the LA of rats treated with the vehicle of aldrin for 12 consecutive days along with the agonist(s)/antagonist(s) of different neurotransmitters on the last day of vehicle of aldrin treatment and the LA of rats treated with the agonist(s)/antagonist(s) of different neurotransmitters (results not shown). Other details are the same as described in the legend of Table 1. Significantly different <sup>a</sup> p < 0.001 using Tukey test for ANOVA

Table 4. Effect of single administration of different combinations of agonist(s) and antagonist(s) of GABAergic, cholinergic and dopaminergic neurons on locomotor activity induced by aldrin treatment for 12 consecutive days in adult male rats

Treatment with agonist(s)/antagonist(s)	Locomotor activity		
	Vehicle of aldrin ± vehicle of agonist(s)/antagonist(s)*	Vehicle of aldrin ± agonist(s)/antagonist(s)**	Aldrin (5 mg/kg/day, po) ± agonist(s)/antagonist(s)
Control	22.56 ± 1.65	22.56 ± 1.65	47.78 ± 3.00 <sup>a</sup>
Bicuculline + atropine	23.04 ± 1.77	24.52 ± 0.76 <sup>a</sup>	42.56 ± 1.97 <sup>a</sup>
Bicuculline + physostigmine	23.31 ± 1.78	10.41 ± 0.77 <sup>a</sup>	39.38 ± 1.87 <sup>a</sup>
L-DOPA + carbidopa + atropine	23.17 ± 1.84	29.48 ± 1.66 <sup>a</sup>	43.39 ± 2.16 <sup>a</sup>
L-DOPA + carbidopa + bicuculline	23.44 ± 1.78	53.78 ± 2.88 <sup>a</sup>	83.96 ± 2.77 <sup>a</sup>
Haloperidol + bicuculline	23.10 ± 1.82	6.27 ± 0.54 <sup>a</sup>	18.03 ± 0.64 <sup>a</sup>
Haloperidol + atropine	23.81 ± 1.62	0.0 <sup>a</sup>	33.07 ± 2.03 <sup>a</sup>
Haloperidol + physostigmine	23.08 ± 1.68	0.0 <sup>a</sup>	17.99 ± 1.25 <sup>a</sup>
L-DOPA + carbidopa + physostigmine	22.90 ± 1.78	11.09 ± 1.08 <sup>a</sup>	33.13 ± 2.08 <sup>a</sup>
L-DOPA + carbidopa + atropine + bicuculline	23.62 ± 1.54	43.46 ± 1.83 <sup>a</sup>	63.90 ± 2.21 <sup>a</sup>
L-DOPA + carbidopa + physostigmine + bicuculline	23.44 ± 1.58	41.83 ± 1.87 <sup>a</sup>	60.43 ± 2.40 <sup>a</sup>

Results are expressed as means ± SEM of 6–8 separate observations. Locomotor activity of control rats (without any vehicle treatment) was 22.54 ± 1.71. The agonist(s)/antagonist(s) were administered on the last day of aldrin treatment. \* No significant difference between the LA of rats treated with the vehicle of aldrin for 12 consecutive days along with the vehicle of agonist(s)/antagonist(s) of different neurotransmitters on the last day of vehicle of aldrin treatment and the LA of rats treated with the vehicle of agonist(s)/antagonist(s) of different neurotransmitters (results not shown). \*\* No significant difference between the LA of rats treated with the vehicle of aldrin for 12 consecutive days along with the agonist(s)/antagonist(s) of different neurotransmitters on the last day of vehicle of aldrin treatment and the LA of rats treated with the agonist(s)/antagonist(s) of different neurotransmitters (results not shown). All other details are the same as described in the legends of Tables 1–3. Significantly different <sup>a</sup> p < 0.001 using Tukey test for ANOVA

stimulation of LA in the presence of aldrin (Tab. 1) was also significantly reduced in the presence of atropine (21%;  $F = 36.24$ ,  $p < 0.001$ ) or physostigmine (38%;  $F = 35.82$ ,  $p < 0.001$ ) (Tab. 2). The aldrin-induced increase in LA was also significantly reduced by haloperidol and atropine (38%;  $F = 32.68$ ,  $p < 0.001$ ) or physostigmine (68%;  $F = 118.32$ ,  $p < 0.001$ ) (Tab. 2). Co-administration of L-DOPA + carbidopa with bicuculline in aldrin treated rats significantly enhanced (43%;  $F = 28.42$ ,  $p < 0.001$ ) the L-DOPA + carbidopa-induced LA in aldrin treated rats (Tabs. 1 and 2). The increase in LA in aldrin treated rat in the presence of L-DOPA + carbidopa and bicuculline was significantly reduced by atropine (28%;  $F = 42.36$ ,  $p < 0.001$ ) or physostigmine (30%;  $F = 54.70$ ,  $p < 0.001$ ) (Tab. 2).

## DISCUSSION

Our recent observation have indicated that aldrin stimulates locomotor activity (LA) by inhibiting regional brain GABAergic activity. Bloomquist and Soderlund, and others [3, 7, 25] have shown that aldrin acts as a GABA<sub>A</sub> receptor antagonist. GABA<sub>A</sub> receptor has been considered as a classical GABA receptor [20] which has been found to be located predominantly in the postsynaptic and dendritic cell [6]. The postsynaptic GABA<sub>A</sub> receptor stimulation with GABA or its agonist opens the Cl<sup>-</sup> ion channel. This influx of Cl<sup>-</sup> ion into the neuronal cell causes hyperpolarization of the postsynaptic membrane and its inhibition [20]. The antagonistic effect of aldrin on GABAergic neurons [14] and the interaction of neuronal GABA with dopaminergic and cholinergic neurons [16, 23, 26] further elicited an interest of the authors to study the antagonistic effect of aldrin on the GABAergic neurons through the interregulation of dopaminergic and cholinergic systems [2, 4, 16, 18, 21, 23, 26] which, if any, may explain the aldrin-induced stimulation of LA under non-tolerant condition.

In the present investigation, the aldrin-induced stimulation of LA and LA of the control rats were found to be reduced by GABA<sub>A</sub> receptor agonist (muscimol), whereas, the antagonist of GABA<sub>A</sub> receptor bicuculline, enhanced the LA of rats under the above experimental conditions suggesting that LA is inversely regulated by central GABA, and aldrin may inhibit central GABA through GABA<sub>A</sub> receptor [10]. Further, a significant increase in LA evoked by dopaminergic receptor agonist (L-DOPA

+ carbidopa) and reduction in LA caused by haloperidol, dopaminergic receptor antagonist in both control and aldrin treated rats (Tab. 1) suggests that central dopaminergic system seems to be involved in the regulation of the LA under the abovementioned conditions. Unlike those central GABAergic and dopaminergic systems, the significant reduction of LA by the treatment with physostigmine, a cholinomimetic agent or atropine (a muscarinic cholinergic receptor blocker) in both control and aldrin-treated rats (Tab. 1) suggests that central cholinergic system may play a role in the regulation of LA in both control and aldrin-treated rats through the interaction with other neurotransmitters. Stevens and others [2, 26] have shown that the activation of dopaminergic system inhibits GABAergic activity through the reduction of cholinergic system. It is known that LA is inversely correlated with the central GABAergic activity [10, 18, 21] and increased with the activation of central dopaminergic system [5]. The attenuation of the decrease in LA induced by haloperidol and atropine or physostigmine and potentiation of increase in LA induced by L-DOPA + carbidopa in aldrin-treated rats (Tab. 1) suggests that aldrin stimulates central dopaminergic system and inhibits cholinergic system.

The antagonistic effect of physostigmine on bicuculline- or L-DOPA + carbidopa-induced increase in LA in aldrin-treated or respective control rats (Tab. 2) indicates that the activation of central cholinergic system activates GABAergic system and hence reduces bicuculline or L-DOPA + carbidopa-induced LA in both aldrin-treated and control rats. On the other hand, treatment with atropine induces presynaptic release of acetylcholine [29] and probably enhances the GABAergic activity *via* stimulating nicotinic receptor [18, 21] and reduces the bicuculline-induced increase in LA in both aldrin-treated and untreated rats. The significant attenuating effect of atropine or L-DOPA + carbidopa-induced increase in LA in both control and aldrin-treated rats (Tab. 2) indicates that atropine in the presence and absence of L-DOPA + carbidopa attenuates the aldrin-induced inhibition of central GABAergic activity. It was also noted in the present study that the inactivation of dopaminergic system with haloperidol greatly enhanced the GABAergic activity *via* the stimulation of cholinergic activity, and as a result of that LA of the control rat was significantly reduced (Tab. 2). The reduction

of LA with co-administration of haloperidol and bicuculline in both control and aldrin-treated rats (Tab. 2) further suggests that inactivation of dopaminergic system greatly enhances the GABAergic activity *via* stimulating cholinergic activity and reduces the bicuculline-induced increase in LA. This may again be supported by the significant haloperidol-induced reduction of LA in the control rats and blockade of the same with atropine or physostigmine (Tab. 2). In contrast to this total blockade of LA in the control rats, the appearance of LA under similar condition of co-administration of haloperidol with atropine or physostigmine to aldrin-treated rats (Tab. 2) suggests that aldrin activates the dopaminergic system and reduces the GABAergic activity through the partial neutralization of haloperidol-induced activation of cholinergic system. This hypothesis may be further supported by a significant increase in LA in aldrin-treated rats and potentiation of aldrin-induced increase in LA with atropine or physostigmine in the presence of L-DOPA + carbidopa and bicuculline (Tab. 2).

High concentrations of GABA are found in the basal ganglia, particularly in the substantia nigra, globus pallidus and nucleus accumbens [19]. Most GABA in the caudate nucleus and putamen is associated with short inhibitory interneurons, while a long-axon GABA-mediated pathway projects from the globus pallidus and striatal complex to the substantia nigra. This GABA-mediated pathway is believed to exert an inhibitory feedback controlling influence on the ascending dopaminergic nigrostriatal projection, and thus to play an important role in extrapyramidal function [15]. The dopaminergic nerve cell bodies are present in the substantia nigra and send axons which terminate in the caudate nucleus – putamen complex. This system and these brain areas are concerned with the integration of the incoming sensory stimuli and the initiation and fine control of movement like locomotor function [15]. Swerdlow and Koob [28] have shown that a GABAergic nucleus accumbens-ventral pallidum projection is believed to serve as the critical first order accumbens efferent pathway underlying the behavioral expression of mesolimbic dopaminergic activity in the rat. They also pointed out that accumbens pallido-thalamic circuitry plays a crucial role in translating the effects of mesolimbic dopaminergic activity to lower motor circuitry responsible for locomotor behavior in the rat.

Thus, from the present pharmacological observation it may be stated that aldrin following its one-day single administration activates dopaminergic system which, in turn, reduces GABAergic activity through the reduction of cholinergic system and increases the LA. As *in vitro* aldrin exerts its antagonistic effect on GABAergic receptors by inhibiting  $\text{Cl}^-$  ion flux [3, 7, 25], the aldrin-induced decrease in the central GABAergic activity *via* the interregulation of dopaminergic and cholinergic systems as revealed from the present study may be suggested to be secondary to the action of aldrin on GABA system in the brain. Like one-day treatment with a single dose (5 mg/kg, *po*), the treatment with aldrin (5 mg/kg/day, *po*) for 12 consecutive days (under nontolerant condition) [10] also enhanced the LA of rats, but to the greater extent (Tab. 3). This may be due to the greater reduction of GABAergic activity in the rats treated with aldrin for 12 consecutive days than that observed with one-day single aldrin treatment [10]. Moreover, the attenuating or potentiating effects of aldrin on agonist(s)/antagonist(s) (either individually or in different combinations)-induced change in LA were greater in rats treated with aldrin for 12 consecutive days (Tabs. 3 and 4) than those observed after a single-day aldrin treatment (Tabs. 1 and 2). These results suggest that the treatment with aldrin for 12 consecutive days increases LA *via* the same mechanism of interaction of GABAergic, cholinergic and dopaminergic neurons as that observed in the animals subjected to one-day aldrin treatment but with a greater potency than that noted in the rats treated with single dose of aldrin.

In this context, it is important to note that the effective toxicity of a compound depends on the resultant effect of the number of simultaneous processes such as rate of penetration, retention of the compound, enzymatic metabolism or biotransformation of compounds and formation of lower or higher amounts of toxic metabolites (by detoxification) and at last elimination through excretion or inert storage of these metabolites in the body [8]. Since aldrin is readily converted to dieldrin [24] and both can be stored within the fat cells in animals like other chlorinated insecticides at a certain saturated level, it may be stated that aldrin-induced increase in LA may be a result of inhibition of central GABA caused by aldrin or its epoxide dieldrin [24] accumulated within the biological system following a single dose of aldrin. In the present study,

the aldrin-induced maximum effect on LA as well as on the brain GABA following 12 consecutive days of its administration may be considered to be an effect of threshold level of aldrin or dieldrin that accumulated within the body following 12 consecutive days of aldrin administration. Further investigations on pharmacokinetics of aldrin will explain the present thought.

Finally, from the results of the present study one may conclude that aldrin under nontolerant condition (both after single dose and 12 consecutive days of treatment) enhances LA by the activation of the central dopaminergic system which, in turn, reduces central GABAergic activity through the reduction of cholinergic system.

*Acknowledgments.* This work was supported by the University Grants Commission (UGC), New Delhi, India, and University of Calcutta, Calcutta, India. Sk. Jamaluddin is a Senior Research Fellow of UGC, University of Calcutta, India.

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*Received: August 16, 2000; in revised form: November 15, 2000.*