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EVIDENCE FOR THE CHANGES IN THE DOPAMINERGIC ACTIVITY AFTER THE SUBACUTE ADMINISTRATION WITH PHYSOSTIGMINE

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ABSTRACT: *Rats were treated with physostigmine acutely and subacutely for 7 days. The duration of tremor, striatal acetylcholinesterase(AChE) activity and the occurrences of dopaminal antagonist-induced catalepsy were measured. During the daily treatment with 0.75 mg/kg of physostigmine, the durations of tremor were markedly reduced. However, the tremors were not occurred in the continuous infusion up to 0.20 mg/kg/hr. Striatal AChE activities were significantly inhibited (82.5%) early hour, but twenty-four hour after daily administration with physostigmine AChE activities were recovered to normal. The striatal AChE activities during the continuous infusion with physostigmine were also significantly inhibited (30-60%). In the study of the occurrence of catalepsy, the dopamine-1 specific antagonist, SCH 23390 (0.1 mg/kg, sc) were administered to the physostigmine pretreated rats. Neither the latency to the on-set nor to the duration of catalepsy were changed in the acutely treated rats. The latency to the on-set was significantly decreased in the daily treated rats, but not in the continuously infused one. However, significant increases in the duration of catalepsy were observed in both daily and continuously treated animals.*

These results indicate that the response to physostigmine is reduced and that to dopamine antagonist is increased after subacute treatment with physostigmine. Further it suggests that the subacute stimulation of cholinergic activity may affect the dopaminergic activity.

Key words: *Physostigmine, subacute treatment, acetylcholinesterase dopaminergic antagonist, catalepsy*

INTRODUCTION

Physostigmine is known to inhibit acetylcholinesterase(AChE) reversibly. As

early as 1946, Koster (1946) studied the inhibition of plasma cholinesterase. The clinical uses and mechanism of action of physostigmine have been reported to improve memory function in patients with Alzheimer's disease (Bartus *et al.*, 1983; Thal *et al.*, 1983). The toxic effects with overdosage of other drugs, such as tricyclic antidepressants (Nattel *et al.*, 1979) and benzodiazepine (Larson *et al.*, 1977) were reported to be reversed by physostigmine. Also it has potential use as a prophylactic agent against organophosphate intoxication (Gordon *et al.*, 1978).

However, physostigmine has a very short half-life (Somani and Khalique, 1986) and the repeated treatments are needed for its uses. The recent improvement of delivery systems increased the use of physostigmine and it has been reported that the continuous infusion of physostigmine might be useful against soman intoxication (Lim *et al.*, 1991). But the continuous infusion of physostigmine induced the changes in the response to physostigmine as well as cholinergic agents (Bhat *et al.*, 1990). Along with that, the studies for the changes in the sensitivity after the intermittent inhibition of AChE are needed for the safe use of physostigmine for long-term treatment.

Fuxe *et al.* (1977) has been shown cholinergic drugs influence other transmitter pathways in the striatum-basal ganglia system, which is rich in monoamine like dopamine as well as in acetylcholine, indicating the existence of cholinergic-dopaminergic interaction.

Furthermore, it has been reported that the irreversible inactivation of AChE (Fernando *et al.*, 1984; Sivam *et al.*, 1983) and the administration of cholinomimetic (Corrodi *et al.*, 1967; Haubrich and Reid, 1972) induced the changes in the dopaminergic activities and in the response to the dopaminergic drug (Davis and Rosenberg, 1981). However little is known about the changes in dopaminergic activity after the intermittent and continuous inhibition of AChE with physostigmine.

The purpose of study is to determine the changes in the sensitivities of cholinergic and dopaminergic responses after the subacute treatment with physostigmine.

MATERIALS AND METHODS

Animals and Chemicals

Male Sprague-Dawley rats (SNU animal house, Seoul, Korea) weighing 200-250 g were used throughout the study. The animals were housed four to a cage with free access to food and water in a temperature-regulated room 12/12 hours light-dark cycle. SCH-23390 was obtained from Research Biochemicals Inc. (Wayland, MA). All other chemicals using for the enzyme assays were obtained from Sigma Chemical Co. (St. Louis, MO).

Treatment Protocol

Two separate experiments were planned as follows:

1) The rats were divided into three groups

Two groups of the rats were subcutaneously injected with physostigmine either acutely or daily for 7 days. The acutely treated rats were sacrificed 30 min, 1 hr,

2 hr or 24 hr after the physostigmine administration of 0.75 mg/kg and 24 hr after the dose of 2.5 mg/kg.

In the daily treated groups, the dose of physostigmine of either 0.25, 0.50 or 0.75 mg/kg were administered daily between 9 and 11 AM for 7 days. These daily treated rats were decapitated either 24 hr following the last injection of 0.75 mg/kg or 30 min after the additional administration of physostigmine in the 7 days treated group. The other group of the rats was implanted with mini-osmotic pumps (Model 2001, Alza Corp., Palo Alto, CA) delivered physostigmine at a rate of either 0.10, 0.15 or 0.20 mg/kg/hr for 7 days. The pumps were implanted under the skin on the backs of the animals after ether anesthesia as described previously (Lim *et al.*, 1989).

The each control groups received similar treatment of saline vehicle.

2) The rats were divided into three groups

The each groups was treated with physostigmine; the acute injection of 0.75 mg/kg, the daily injection of 0.75 mg/kg for 7 days and the continuous infusion of 0.15 mg/kg/hr for 7 days. At twenty four hours after the last injection(s), both the control and the treated rats were treated with SCH 23390 of 0.1 mg/kg, s.c., dopamine-1 specific antagonist (Consolo *et al.*, 1988) and the drug-induced catalepsy was measured.

Determination of Gross Behavioral Changes

Doses of physostigmine were given to rats subcutaneously and they were monitored over a period of 2 hr for the occurrence of gross behavior : salivation, lacrimation, tremors and mortality. Expecially the occurrence of tremors after daily dose of 0.75 mg/kg were observed every 5 min after the administration and recorded according to the procedure of Fernando *et al.* (1984) and Weinstock *et al.* (1980). The time for the on-set of tremors was defined as the time starting the distinct slow tremor of head (the scale 1) and that for the duration of tremor was as the time interval between the start and the disappearance of the intensive fast tremor (the scale 3).

Determination of Cholinesterase and Acetylcholinesterase Activity

Whole blood was used for determination of total cholinesterase activity(ChE). An appropriate amount of blood at 1, 3 and 7 days after the mini-osmotic pump implantation (0.10 mg/kg/hr), 24 hr after 1 and 3 days daily administration of drugs and 30 min after the additional dose in the 7 days daily treated groups was collected from the tail and immediately assayed according to the method of Ellman *et al.* (1961). Upon sacrificing the rats in the indicated time, the striata was dissected out according to the method of Glowinski and Iversen (1966). Striatum were homogenized in ice-cold sodium phosphate buffer (0.1 M, pH 8.0) at a concentration of approximately 20 mg wet weight/ml buffer.

The striatal acetylcholinesterase(AChE) activity was also measured according to the method of Ellman *et al.* (1961). Although the method of Ellman *et al.* (1961) does not distinguish between AChE and butyrylcholinesterase(BuChE), BuChE activity in brain is very low and contributes a negligible amount to the AChE activity when acetylthiocholine is used as substrate (Hobbiger and Lancaster,

1971). The AChE activity was expressed as nmol acetylthiocholine iodide hydrolyzed /mg of protein /min.

The protein content of tissue homogenates was determined by the method of Lowry *et al.* (1951) using bovine serum albumin as a standard.

Measurement of Catalepsy

Catalepsy was assessed by the bar method as described by Meller *et al.* (1985) with minor modification. The front paws were gently placed on a horizontal metal bar 1.00 cm in diameter suspended 10 cm above the work surface. The front paws of each rat after the administration of SCH 23390 was placed in the horizontal bar to record the time of the on-set and the termination of catalepsy.

The latency to the on-set and the duration of catalepsy was recorded for placement of both paws on the floor up to a 600 seconds.

Statistics

The statistical significance of differences were determined using Student's t-tests.

RESULTS

In Vitro Determination of the Potency and Stability of Physostigmine

The potency of physostigmine was determined in terms of the inhibition of cortical AChE activity and IC_{50} was 0.082 μM.

There were no significant changes in potency of physostigmine even after 7 days of incubation at 37°C in the saline (109.4± 7.4% for 1, 101.1± 4.4% for 3 and 97.7± 4.8% for 7 days incubation). These results indicate that physostigmine is faily stable in the saline.

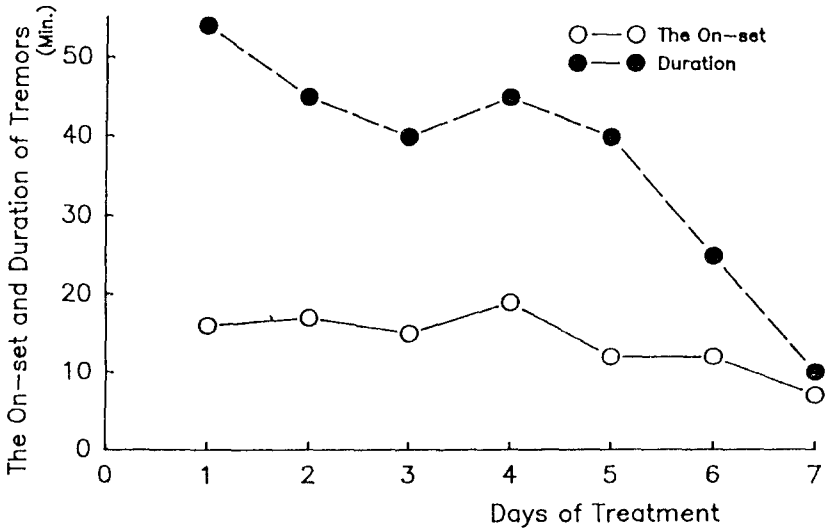

Figure 1. The latency to the on-set and the duration of tremors following daily administration with physostigmine (0.75 mg/kg). Values are means of two observations.

Effects of Acute and Subacute Administration of Physostigmine on the Gross Behavioral Activity

Acute administration of physostigmine with the dose of 0.75 mg/kg induced the weak and strong tremors and the increased doses of physostigmine to 2.5 mg/kg showed the parasympathetic overactivities consisting of salivation, lacrimation, and severe tremors and mortalities. On the subacute administration of physostigmine with the dose of 0.75 mg/kg, the latency to onset in the weak tremors and the duration in the strong tremors were shown in Fig. 1 and their occurrances were considerably shortened in the 7 days treated animal groups. However, on the groups in the continuous infusion of physostigmine for 7 days, the toxic symptoms such as tremors and mortalities, were not appeared.

Table 1. Effects of acute administration of physostigmine on AChE activities in the rat striatum

Dose/Time	Acetylcholinesterase activities (nmol/min/mg protein)				
	Control	30 min	1 hr	2 hr	24 hr
0.75 mg/kg	218.1± 1.9	38.2± 1.9** (82.5)	45.0± 3.5** (79.4))	95.2± 12.4** (56.4)	220.0± 3.3
2.5 mg/kg	242.4± 2.6	–	–	–	248.2± 1.9

[1] Rats were sacrificed the indicated time after physostigmine administration.
[2] The values are the mean± S.E. of four determinations.
[3] The parentheses are the percent inhibition from the control value.
[4] **P<0.001 compared with the corresponding control value.

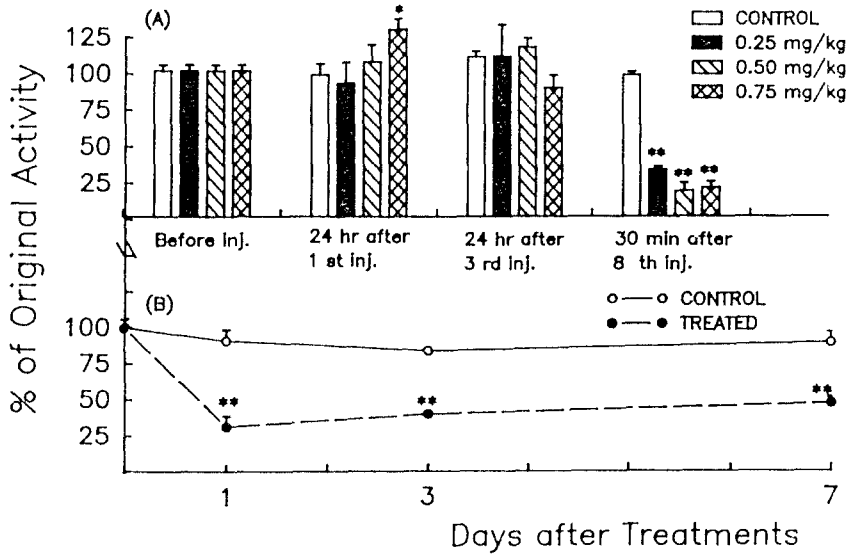

Figure 2. Changes in blood cholinesterase activities during physostigmine treatments. Rats were treated with physostigmine either (A) daily administered (0.75 mg/kg) or (B) continuously infused (0.10 mg/kg/hr). Blood were collected from the tail and cholinesterase activities were measured. The enzyme activity are compared to their original activity. Values are mean± S.E. for four determinations. *P<0.05, **P<0.01 compared with the corresponding control.

Table 2. Effects of subacute administration of physostigmine on AChE activities in the rat striatum

Dose/Methods	Acetylcholinesterase activities (nmol/min/mg protein)				
	Daily injection			Continuous infusion[c]	
Control	260.1± 10.5[a]	220.5± 6.7[b]	Control	225.6± 8.4	235.6± 5.8
0.25 mg/kg	73.2± 8.9** (71.8)	–	0.1 mg/kg/hr	160.4± 5.6** (28.9)	–
0.50 mg/kg	59.5± 10.1** (77.2)	–	0.15 mg/kg/hr	–	85.6± 9.6** (64.6)
0.75 mg/kg	48.0± 3.8** (81.5)	218.0± 8.5	0.2 mg/kg/hr	–	81.2± 4.4** (65.5)

[1] Rats were sacrificed [a] 30 min after 8th and [b] 24 hr after 7th administration of physostigmine and [c] 7 days after the continuous infusion of physostigmine via mini-osmotic pump as indicated doses.

[2] The values are the mean± S.E. of four or five determinations.

[3] The parentheses are the percent inhibition from the control value.

[4] **$P<0.001$ compared with the corresponding control value.

Effects of Acute and Subacute Administration of Physostigmine on AChE Activities in Brain and Blood

The striatal AChE activities after acute administration with physostigmine were summarized in Table 1. Thirty min after an acute administration of physostigmine (0.75 mg/kg), AChE activities were significantly reduced (17.5%) in striatum and then their activities were gradually and rapidly recovered. Twenty four hour after the administration of high dose of physostigmine (2.5 mg/kg), striatal AChE activities were essentially the same as the level of the control.

The blood ChE activities during daily and continuous administration of physostigmine were shown in Fig. 2. Twenty-four hour after 1st and 3rd administration in various dose of physostigmine, blood ChE activities were the same as the control level. And thirty min after the additional dose in the 7 days treated groups, the ChE activities in blood were significantly inhibited and their inhibited rates were 57, 81 and 79% of the control, respectively (Fig. 2A). However, the ChE activities during the continuous infusion with physostigmine (0.1 mg/kg/hr) were 35-52% of the control in blood (Fig. 2B).

The striatal AChE activities after daily and continuous administration of physostigmine were summarized in Table 2. The striatal AChE activities 30 min after the additional dose of physostigmine in the 7 days treated rats were significantly inhibited and their activities were 28.2, 21.8 and 18.5% of the control levels, respectively. However, before the additional dose (0.75 mg/kg), enzyme activities were essentially the same as the control.

After the 7 days infusion in the dose of physostigmine with 0.1, 0.15 and 0.2 mg/kg/hr, the AChE activities were significantly inhibited and their inhibitions were 28.9, 64.6 and 65.6% of the control, respectively.

Effect of Acute and Subacute Administration of Physostigmine on SCH 23390-Induced Catalepsy

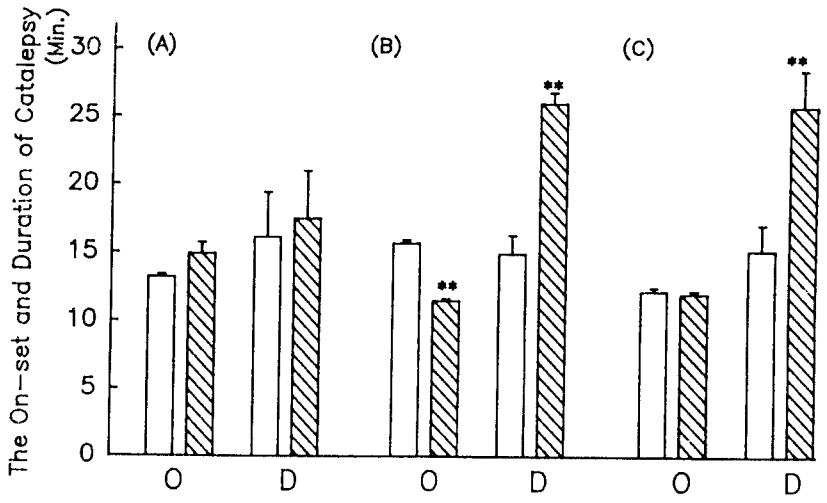

Figure 3. The latency to the on-set (O) and the duration (D) of SCH 23390-induced catalepsy in the various physostigmine treated rats.
Rats were treated with physostigmine with a) acutely (0.75 mg/kg), b) daily (0.75 mg/kg) and c) continuously infused (0.15 mg/kg/hr) for 7 days. The catalepsy was induced by the injection of SCH 23390 (0.1 mg/kg, sc.) and the occurred times were recorded in individual rats. The open and the slashed bar are saline and physostigmine pretreated groups, respectively. Values are mean± S.E. for three to five rats. **P<0.01 compared with the corresponding control.

The latency of on-set and the duration of catalepsy after the administration of SCH 23390 in the physostigmine treated groups were shown in Fig. 3. There were not significantly different in the acutely treated rats with physostigmine (0.75 mg/kg). However, the latency of the on-set to the SCH 23390-induced catalepsy were significantly decreased in the daily 7 days treated animals (15.47± 0.25 vs. 11.53± 0.16 min) and the durations were significantly increased in both the daily 7 days treated animals (15.01± 1.35 vs. 26.05± 0.84 min) and the continuously infused animals with physostigmine of 0.15 mg/kg/hr (15.30± 1.91 vs. 25.92± 2. 70 min).

DISCUSSION

The present results demonstrate that the subacute administration of physostigmine produce the changes in the responses to physostigmine as well as dopamine antagonist, SCH 23390. This changes is evident by decreasing the duration of tremors after the additional dose with physostigmine and by increasing the duration of catalepsy after the administration of SCH 23390. These results suggest that the stimulation in the cholinergic nerve activities may induce the changes in the dopaminergic nerve activities.

It has been reported that the long-term administration of organophosphate induced subsensitivity to acetylcholine and there was a significant decrease in the numbers of muscarinic and nicotinic receptors (Ehlert *et al.*, 1980; Lim *et al.*, 1987; Russel *et al.*, 1981). However, Yamada *et al.* (1983) reported that the

characteristic of muscarinic receptors had been not changed after the subacute administration of physostigmine. But the present results reveals that the duration of tremors in the subacutely treated group with physostigmine is shorter than that in the acutely treated one without the difference in the inhibiton rate of AChE between two treatments. This suggest that the subacute administration of physostigmine may develop the tolerance to physostigmine. It has been reported that the cortical and striatal acetylcholine contents were decreased after the administration of physostigmine (Bartolini *et al.*, 1973) and the subacute administration of organophosphate (Lim *et al.*, 1987). Recently, Bhat *et al.* (1990) has reported that the continuous infusion with physostigmine developed marked tolerance to physostigmine and reduced the nicotinic receptors in the mice brain. Lim *et al.* (1989) has reported that the continuous infusion with the high dose of physostigmine on guinea pigs showed tremors and more than 40% mortalities. However, the present study shows that there were no mortalities. The discrepancy might be due to the different sensitivity of species to physostigmine : the guinea pigs and rats.

It has been reported that the biological half-life of physostigmine is very short in plasma and brain and it easily penetrates the blood brain barrier (Somani and Khalique, 1986). Somani *et al.* (1991) reported that physostigmine has a properties with a rapid distribution followed by a faily rapid elimination. Thus the rapid recovered AChE activity after an acute treatment might be due to rapid clearance of this drug.

Although the dose-dependent inhibition of AChE in the subacutely treated animals are shown, the small differences in the AChE activities during the daily and high dose of continuous treatment are remained to be intensively studied.

It has been suggested that dopaminergic and cholinergic mechanisms interact in a delicate way to maintain the normal function of striatum (Anden *et al.*, 1966). The striatal dopaminergic activity has been reported to be changed after the acute and subacute administration of the central cholinomimetics and the changes in the dopaminergic activity were suggested to be counteracted with the changes in the cholinergic activity (Fernando *et al.*, 1984; Sivam *et al.*, 1983). Several investigators have reported that the turnovers of brain dopamine after an acute administration of organophosphate and cholinergic agonist such as, diisopropylfluorophosphate and oxotremorine, were increased (Fernando *et al.*, 984;; Javoy *et al.*, 1975). Davis and Rosenberg (1981) reported that organophosphate pretreated animals induced the behavioral supersensitivity to apomorphine, dopamine agonist, in the early time point but not 24 hours after the administration of diisopropylfluorophosphate. However, it has been reported that the subacute administration of organophosphate induced either the decrease or the increase in the dopamine turnovers in the rat striatum (Fernando *et al.*, 1984; Freed *et* al., 1976). Also Sivam *et al.* (1983) has reported that the numbers of dopaminergic receptors were increased after the long-term administration with organophosphates. The significant increase in the SCH 23390-induced catalepsy as our results indicate that the intermittant as well as the continuous inhibition of AChE activity after subacute administration of physostigmine induce the supersensitivity to dopamine antagonist. Although biochemical studies should help

to elucidate the mechanisms for the changes in the dopaminergic nerve sensitivity after the subacute administration, the findings of the present study suggest that the stimulation of cholinergic nerve may alter the sensitivity of dopaminegic nerve system.

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