EFFECT OF ACUTE PHYSOSTIGMINE AND VERAPAMIL TREATMENT ON AGGRESSIVE AND DEPRESSIVE BEHAVIOR IN RATS WITH LESIONED NUCLEUS BASALIS MAGNOCELLULARIS

Miroljub Popović[1,2], Natalija Popović[2], Dubravko Bokonjić[3], Silva Dobrić[3], Nenad Ugrešić[4] and Vladimir S. Kostić[5]

[1]Departamento de Ciencias Morfológicas y Psicobiología, Facultad de Medicina, Campus Universitario de Espinardo, Universidad de Murcia, 30100 Murcia, Spain;
[2]Immunology Research Center "Branislav Janković", Vojvode Stepe 458, Belgrade FR Yugoslavia;
[3]Medical Department, Military Technical Institute, Katanićeva 15, Belgrade, FR Yugoslavia;
[4]Department of Pharmacology, Faculty of Pharmacy, Vojvode Stepe 450, Belgrade, FR Yugoslavia;
[5]Institute of Neurology, Clinical Center of Serbia, Dr. Subotića 6, Belgrade, FR Yugoslavia

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SUMMARY

In order to investigate the effects of physostigmine and verapamil on aggressive (test of foot shock induced aggression) and depressive (learned helplessness test) behavior, ten days after bilateral lesions of the nucleus basalis magnocellularis (NBM), adult male Wistar rats were acute treated (30 min before the test) with physostigmine (0.045, 0.060 and 0.075 mg/kg, s.c.) or verapamil (1.0, 2.5, 5.0 and 10.0 mg/kg, s.c.). Physostigmine in dose of 0.075 mg/kg and verapamil in doses 2.5 and 5.0 mg/kg significantly prolongated the escape latency period in the learned helplessness test and thus produced a consolidation of depressiveness in NBM-lesioned rats. In contrast to that, there was no restitution of aggressive behavior in NBM-lesioned rats treated with both drugs. It could be concluded that both physostigmine and verapamil exerts a significant influence on depressive, but not on aggressive reaction in an animal model of Alzheimer's disease.

KEY WORDS: Alzheimer's disease, aggression, depression, physostigmine, verapamil, rats

INTRODUCTION

Alzheimer's disease (AD) is a neurodegenerative disorder

producing deficits of cognitive and noncognitive functions. Firstly, cognitive disturbances were considered as primary features of AD, but lately several studies have revealed that noncognitive disturbances such as aggression (1-12) and depression (2,3,12-17) represent important characteristics of AD. Namely, most of the studies suggest that aggressive and depressive behavior occur in between 24 and 63% of patients with AD (2,18-22). Moreover, these disturbances are a serious problem for families or hospital staff which take care about AD patients (4,17,23-25).

The etiology of aggressive and depressive symptoms in AD patients is still unclear. There are several possible neurochemical substrates for aggression in AD patients (26). However, the most important changes are related to the decrease of serotoninergic activity (27-30), which is highly correlated with aggressive behavior in AD patients (31,32). On the other hand, the degree of depression in AD patients is in correlation with loss of norepinephrine neurons in the locus coeruleus (33).

The treatment of the behavioral disturbances in AD patients requires not only drug administration, but also to maintain a consistent life style and environment. Therefore, pharmacological (34-39) and nonpharmacological approaches (39-44) were employed with the aim of treat aggression and depression in AD patients.

The antiaggressive drug therapy was based on the treatment with benzodiazepines (9,26), neuroleptics (45-48), beta-adrenergic-blocking agents (49,50), serotonergic agents (51,52,53), lithium (49), antiandrogens (36) as well as anticonvulsants (37,54-58).

For the treatment of depressive symptoms, MAO inhibitors (whose pharmacological activity is aimed to treat the reduced norepinephrine levels (59-62)) and psychostimulants (63,64) has been mainly used.

Our recent study suggests that bilateral electrolytic lesions of the nucleus basalis magnocellularis-NBM in rats (an animal model of AD), induced low level of aggressive and depressive behavior (65). Having in mind that the increase of central cholinergic activity with physostigmine and the regulation of altered intracellular calcium homeostasis by verapamil could ameliorate some cognitive and noncognitive disturbances in an experimental model of AD (66,67), the present study was done to investigate the effect of these two drugs on aggressive and depressive behavior in NBM-lesioned rats.

MATERIALS AND METHODS

Experimental animals

Experiments were carried out on adult male Wistar rats (250-280 g). The rats were housed in groups of 5 to a plastic cage in standard laboratory conditions (room temperature of 20 ± 1°C, 30% humidity, 12/12 hr light/dark cycle) with food and tap water available ad libitum. All tests were performed during the light period.

The rats were divided in the following groups: NBM-lesioned rats treated with saline, physostigmine or verapamil. Nine rats were assigned to each group within each task.

Surgery

The rats were anesthetized with sodium pentobarbital (35 mg/kg ip) and following bilateral electrolytic NBM lesions were made as described in previous studies (65-67). The recovery period after operation was ten days.

Foot shock aggression

Paired rats were placed in a plastic box (48 x 21 x 22.5 cm) with an electrifiable grid floor. The rats were exposed to ten 5 s shocks (3 mA) with shock-free intervals of 25 s for a total test time of 5 min (65).

The fighting positions were taken into account only when they were exhibited during the shock-free intervals. A fighting position occurred when both animals assumed the upright boxing posture, standing face to face, and pushed with their forepaws. We considered the number of aggressive pairs, the number of shocks required to elicit the first attack (latency period), the number of fighting positions during the whole test and degree of aggression. The degree of aggression was arbitrarily graded from 0 to 4 as described by Popović et al. (65).

Learned helplessness

Inescapable shocks sessions and escape testing were carried out in plexiglas boxes (24 x 10.5 x 11 cm) and plexiglas shuttle-boxes (48 x 21 x 22.5 cm). Each of these boxes were housed in sound-attenuated chambers.

The rats were individually placed in the plexiglas box for 1 hr period. During this time rats received 60 inescapable 5 s (3 mA) shocks. The intertrial interval was 60 s in duration (65).

Twenty four hours later, the preshocked rats were individually placed in the shuttle boxes and exposed to 5 s (3 mA) shocks every 60 s. The shocks were delivered until animals made the escape response (crossing on the other side of shuttle-box). The number of shocks delivered before the animal made the correct escape response (i.e. escape latency) was used as a parameter for assessment of the animal depression status (65).

Drugs

Solutions of physostigmine salicylate (BDH, Chemicals LTD, England) and verapamil hydrochloride (Sigma, St. Louis, MO) were prepared freshly daily in light-tight bottles and diluted with physiological saline, so that all doses were administered in a constant volume of 1 ml/kg body weight. Physostigmine (0.045, 0.060

and 0.075 mg/kg) and verapamil (1.0, 2.5, 5.0 and 10.0 mg/kg) as well as physiological saline (1 ml/kg) were given subcutaneously to the NBM-lesioned rats, 30 min before the tests.

Histology

The position of NBM-lesions were examined as described in previous studies of our group (65-67).

Statistics

All data are expressed as means ± S.D. Statistical comparison was performed using the Kruskal-Wallis anaylisis of variance test, the Mann Whitney U-test and the t-test proportion. Differences were accepted as statistically significant when $p < 0.05$.

RESULTS

Our results shown that there were no differences in the number of aggressive pairs, the number of fighting position, the latency up to first fighting position as well as the aggression scores between saline, physostigmine and verapamil treated NBM-lesioned rats (Table I and II).

In comparison to saline treated NBM-lesioned rats, the mean escape latency was significantly higher in NBM-lesioned rats treated with physostigmine (0.075 mg/kg) and verapamil (2.5 and 5.0 mg/kg) (Table III and IV).

DISCUSSION

A recent study of our group suggests that besides cognitive deficits, noncognitive behavioral disturbances exist in an experimental model of AD (65). Although most of the clinical studies had shown the existance of aggression and depression in AD patients (1-17), the present study had shown that the NBM-lesioned rats express less aggressivness in the foot shock-induced aggression test and less depressiveness in the learned helplessness test. However, even these data do not correlate with data obtanined from AD patients, they are in correlation with the results obtained in experimental studies of aggression and depression. Namely, it has been established that both mice and rats, after pharmacological induced cholinergic deprivation are less aggressive (68-70) and less depressive (71,72). Having in mind the importance of the cholinergic and monoaminergic systems in the regulation of aggression and depression (73-79), the obtained differences could be related to differences in the degree of the cholinergic/monoaminergic system imbalance in NBM-lesioned rats and AD

Table I. Effect of physostigmine (PHY) on foot shock induced aggression in NBM-lesioned rats

Treatments (mg/kg)	Aggressive pairs (%)	Number of shock to elicit the first fighting position	Number of fighting positions	Aggression scores
SALINE	11.11	9.67 ± 1.00	0.33 ± 1.0	0.22 ± 0.67
PHY (0.045)	0.0	0.0	0.0	0.0
PHY (0.060)	22.22	8.33 ± 3.39	0.22 ± 0.44	0.22 ± 0.44
PHY (0.075)	11.11	9.22 ± 2.33	0.11 ± 0.33	0.11 ± 0.33

The data are present as mean ± S.D.

Table II. Effect of verapamil (VER) on foot shock induced aggression in NBM-lesioned rats

Treatments (mg/kg)	Aggressive pairs (%)	Number of shock to elicit the first fighting position	Number of fighting positions	Aggression scores
SALINE	11.11	9.67 ± 1.00	0.33 ± 0.10	0.22 ± 0.67
VER (1.0)	22.22	8.78 ± 2.73	0.22 ± 0.44	0.22 ± 0.44
VER (2.5)	11.11	9.56 ± 1.33	0.11 ± 0.33	0.11 ± 0.33
VER (5.0)	33.33	8.44 ± 3.09	0.44 ± 0.73	0.44 ± 0.77
VER (10.0)	22.22	9.11 ± 1.83	0.22 ± 0.44	0.22 ± 0.44

The data are present as mean ± S.D.

patients.

Table III. Effect of physostigmine (PHY) on depression in NBM-lesioned rats in learned helplessness test

Treatments (mg/kg)	Mean escape latency
SALINE	1.89 ± 1.96
PHY (0.045)	2.33 ± 1.94
PHY (0.060)	2.67 ± 2.18
PHY (0.075)	4.11 ± 3.82*

The data are present as mean ± S.D. *p < 0.05 vs saline treated NBM-lesioned rats.

Table IV. Effect of verapamil (VER) on depression in NBM-lesioned rats in learned helplessness test

Treatments (mg/kg)	Mean escape latency
SALINE	1.89 ± 1.96
VER (1.0)	2.78 ± 2.28
VER (2.5)	4.44 ± 3.61*
VER (5.0)	4.44 ± 3.91*
VER (10.0)	3.77 ± 2.99

The data are present as mean ± S.D. *p < 0.05 vs saline treated NBM-lesioned rats.

Besides the above mentioned data, recent findings obtained in our laboratory suggest that learning and memory impairment (66) as well as open field behavior (67) could be, partly or completely, ameliorate by acute physostigmine and verapamil treatment, respectively. The present data suggest that both drugs, physostigmine (0.075 mg/kg s.c.) and verapamil (2.5 and 5.0 mg/kg s.c.) regulate depressive, but not aggressive behavior in NBM-lesioned rats.

The regulation of depressive behavior by physostigmine it was expected since it is well known that its administration to intact animals and humans produce depression (74,80). On the other hand, several clinical data suggested that calcium channel blockers, mainly verapamil, have a beneficial antidepressant effect (81-83). In contrast to clinical data, preclinical studies indicate that verapamil

express an antidepressant effect only in some models of animal depression. Thus, verapamil reduced depressiveness in the rat forced swim test (84) but not in the mouse behavioral despair and rat learned helplessness test (85,86). In contrast to that in NBM-lesioned rats verapamil induced a significant prolongation of escape latency and by this way induced depression. Having in mind the cognitive hypothesis of learned helplessness induced depression (87), it may be possible that the obtanied depression after verapamil treatment be related to the reduction of cognitive deficits in NBM-lesioned rats. Namely, our recent studies showed that verapamil in doses of 2.5 and 5.0 mg/kg significantly improved learning and memory processes in active avoidance test (66). In contrast to this, physostigmine in dose of 0.075 mg/kg, which failed to improve learning and memory in active avoidance test (66), significantly increased the escape latency in the learned helplessness test. Having in mind that several studies suggest that the behavioral disruption in learned helplessness test is due to a variety of neurochemical changes invoked by stress (71,88-91), it may be possible that the effect of physostigmne be related to the regulation of stress mechanisms activated during the learned helplessness test.

Although some studies suggest that physostigmine could potentiate the aggressive behavior in foot shock-induced aggression as well as in isolation-induced aggression in mice (69,70,92) some other studies indicated that physostigmine suppress the amphetamine- and apomorphine-induced aggressivness in mice and rats, respectively (93,94) as well as foot shock induced aggression in rats (95). However, it was found that verapamil decrease apomorphine-induced aggression in rats (96) as well as foot shock induced aggression in mice (92). However, the present study shown that neither verapamil or physostigmine in the used doses, were not able to improve the aggressive behavior in NBM-lesioned rats. Probably the anti-aggressive effect that both drugs express in intact rats muy be the reason for unchagable aggressive behavior in NBM-lesioned rats after the treatment with physostigmine or verapamil.

In the light of the present results it may be concluded that both the regulation of calcium homeostasis by verapamil as well as the neurochemical effects of physostigmine and verapamil, could play an importat role in the regulation of depressive behavior, but not of

aggressive behavior in NBM-lesioned rats. Therefore, further studies are needed to find the most effective way to treat the aggressive behavior impairment in an animal model of AD.

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