https://doi.org/10.31925/farmacia.2019.2.14 **ORIGINAL ARTICLE**

THE BEHAVIORAL STUDY OF THE EFFECTS OF ATRISTAMINE ON THE SEROTONIN, DOPAMINE AND NOREPINEPHRINE NEUROTRANSMITTER SYSTEMS IN MICE

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Abstract

The present work reports the behavioural study of the effects on the serotonin, dopamine and norepinephrine neurotransmitter systems of 2-methyl-3-(phenylaminomethyl)-1H-quinolin-4-one (atristamine), a novel promising substance that exhibits excellent antidepressant effect combining with additional valuable neurotropic properties. The results indicate that the acute administration of atristamine increases the responsiveness of the adrenergic system. It has been proven by enhancement of clonidine-induced aggressive behaviour and attenuation of clonidine-induced depression in mice. It has been shown that atristamine attenuates haloperidol-induced catalepsy in mice, but this effect does not associate with MAO and COMT inhibition. A mild modulating effect of atristamine on the serotonin neurotransmitter system was discovered using 5-HTP induced head-twitch response in mice. It has been concluded that the antidepressant effect of atristamine may be explained predominantly by its complicated influence on the serotonin, dopamine and norepinephrine neurotransmitter systems.

Rezumat

Lucrarea de față prezintă studiul comportamental al efectelor asupra sistemelor de neurotransmițători serotonină, dopamină și norepinefrină ale 2-metil-3- (fenilaminometil) -1H-chinolin-4-onă (atristamina), o substanță promițătoare care prezintă un efect antidepresiv pronunțat, alături de alte proprietăți neurotropice. Rezultatele indică faptul că administrarea acută a atristaminei mărește capacitatea de reacție a sistemului adrenergic. Aceasta a fost dovedită prin creșterea comportamentului agresiv și atenuarea depresiei induse de clonidină la șoareci. S-a demonstrat că atristamina atenuează catalepsia indusă de haloperidol, dar acest efect nu se asociază cu inhibarea MAO și COMT. Un efect modulator al atristaminei asupra sistemului serotoninergic a fost demonstrat utilizând un model experimental cu 5-HTP. S-a concluzionat că efectul antidepresiv al atristaminei poate fi explicat prin efectul său asupra serotoninei, dopaminei și norepinefrinei.

Keywords: 2-methyl-3-(phenylaminomethyl)-1H-quinolin-4-one, atristamine, clonidine-induced behaviour, haloperidol-induced catalepsy, head twitches response, L-DOPA induced hyperactivity

Introduction

Mental disorders comprise a broad range of issues manifested with different symptoms. Generally, they may be characterized as a complex combination of abnormal thoughts, emotions, behaviour and relationships with others [26]. Mental health problems are one of the main causes of the burden of disease worldwide [28]. According to the Global Burden of Disease Study (2010), depression and anxiety are the most predominant mental health problems worldwide [29].

Treatment of mental disorders often includes the use of drugs. Drugs can play a key role in treating many mental disorders and conditions. Antidepressants, anti-anxiety drugs, antipsychotics, stimulants and mood stabilizers are often used for these purposes [4]. The key commonality of their mechanisms of action is the regulative effects on the neurotransmitter systems in the CNS. All of them are effective and allow correcting even severe conditions. However, the long-term administration of these drugs may cause development of serious adverse effects. As a result, many patients have to discontinue taking drugs within the first year. Thus, notwithstanding the abundance of modern medicines, the goal to develop an "ideal drug" has not been reached yet and is still of great interest.

Unfortunately, absolutely new or innovative drugs for relieving mental disorders do not often appear. It is not a simple task to find insufficiently studied and, at the same time, promising classes of chemical compounds.

The new substance – 2-methyl-3-(phenylamino-methyl)-1H-quinolin-4-one (know under the name of atristamine) is now extensively studied in the National University of Pharmacy (Kharkiv, Ukraine) (Figure 1).

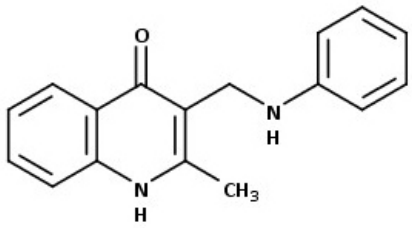

Figure 1.
The structural formula of 2-methyl-3-(phenyl-aminomethyl)-1H-quinolin-4-one (atristamine)

Atristamine exhibits excellent antidepressant properties on the experimental models of depression [19, 22, 23]. It also possesses the anti-amnesic, cerebro-protective, antihypoxic, actopropective and analgesic activities [14-18].

Earlier studies using ELISA methods have been found that a reliable decrease in the concentration of serotonin (–16.8%, $p < 0.05$) was consistent with the increased levels of dopamine (+22.0%) and epinephrine (+13.0%) in the brain of mice after administration of atristamine in the dose of 100 mg/kg bw [24]. Thus, pharmacological effects of atristamine were associated with these changes. But the behavioural equivalents and contributions of each neurotransmitter system have not been evaluated yet.

Different effects of atristamine on the neuro-transmitter systems of the brain have been already discussed in one form or another in previous studies. For example, it has been shown that atristamine in the dose of 100 mg/kg bw has an insignificant influence on the GABA-ergic system (on the models of thiosemicarbazide-induced seizures and thiopental-induced narcosis in mice), glycine-ergic structures (on the model of strychnine-induced seizures in mice) and mildly modulates the purinergic system (decreases the anxiogenic effect of caffeine in the open field test in mice) [20].

Furthermore, antagonism with reserpine has been proven for atristamine on the model of depression in rats [22]. It is well known that reserpine when given to various animal species produces a characteristic set of behaviours, including ptosis, hypothermia, huddling, inactivity, social withdrawal, and sedation. These and other behavioural changes have been likened to human depression. But, reserpine has so many different neurochemical effects that it is difficult to determine which mechanism is associated with which behaviours [10].

The present paper aimed to determine the inter-relations between the effects of atristamine and serotonin, dopamine and norepinephrine neuro-transmitter systems *in vivo* using the corresponding pharmacological analysers.

Materials and Methods

Animals

Adult random-bred male albino mice with the body weight of 18 - 24 g were included in the present study. The animals were obtained from the *vivarium* of the Central Research Laboratory (National University of Pharmacy, Kharkiv, Ukraine). Experiments were carried out in accordance with "Directive 2010/63/EU of the European Parliament and of the Council of 22 September 2010 on the protection of animals used for scientific purposes". The mice were kept in standard polypropylene cages at 20 - 26°C and 50% humidity in a well-ventilated room in the 12 h light/dark cycle with free access to food and water [3]. All experimental protocols were approved by the Bioethics Commission of the National University of Pharmacy.

Drugs and Chemicals

Atristamine (2-methyl-3-(phenylaminomethyl)-1H-quinolin-4-one) was synthesized at the Medicinal Chemistry Department of the National University of Pharmacy (Kharkiv) as reported [30]. In all experiments atristamine was administered intra-gastrically (i.g.) as an aqueous fine suspension stabilized with Tween 80. The mice from the vehicle control groups received saline i.g. by the similar scheme. The volume of liquid that the animals in all groups received was similar and equalled to 0.1 mL/10 g bw.

Clonidine hydrochloride, L-3,4-dihydroxyphenyl-alanine (L-DOPA), 5-hydroxytryptophane (5-HTP) were obtained from Sigma Chemical Company (USA). Haloperidol was used in the form of the solution for injection 5 mg/mL (trade name "Halopril", Zdorovye Narodu, Kharkiv, Ukraine).

Imipramine hydrochloride was chosen as the reference drug for the model of clonidine-induced aggressive behaviour to assess the effect of acute administration of classical tricyclic antidepressants on aggressive response of mice. Imipramine was used in the form of the solution for injection, 25 mg/2 mL (trade name "Melipramine", EGIS Pharmaceuticals PLC, Hungary).

Solutions were prepared *ex tempore* on the days of the experiment by dissolving drugs in physiological saline (0.9% NaCl) or diluting with it. Pharmacological analysers and reference drug solutions were introduced intraperitoneally (i.p.).

Pharmacological tests

All behavioural experiments were performed between 9:00 and 16:00 h in a quiet room, at 23 - 26ºC.

Clonidine-induced aggressive behaviour

For this test, animals were divided into 4 groups:

Group 1 - vehicle control group of animals treated with saline (i.g.) before i.p. injection of saline (10 mL/kg bw), n = 8. This group was formed to check the aggressiveness of mice without chemical induction.

Group 2 - positive control group of animals introduced with saline (i.g.) before i.p. injection of clonidine solution, n = 8.
Group 3 - group of animals treated with atristamine (100 mg/kg bw, i.g.) before i.p. injection of clonidine solution, n = 8.
Group 4 - group of animals treated with imipramine (25 mg/kg bw, i.p.) before i.p. injection of clonidine solution, n = 8.
Clonidine was injected in the dose of 10 mg/kg bw 1 h after introduction of atristamine, imipramine or saline. Immediately after that groups of 2 mice each were placed together in glass cylinders and were observed for 1 h. Aggression was expressed as the number of biting attacks among 2 mice within 1 h [11].

Clonidine-induced depressive behaviour

Animals were randomly assigned into 3 groups:
Group 1 - vehicle control group treated with saline (10 mL/kg bw, i.g.) before saline i.p. injection (10 mL/kg bw), n=7.
Group 2 - animals introduced with saline (10 mL/ kg bw, i.g.) before clonidine injection (i.p.), n = 8.
Group 3 - animals pretreated with atristamine (100 mg/kg bw, i.g.) before clonidine injection (i.p.), n = 8.
One hour after the single administration of atristamine or saline, clonidine was injected i.p. in the dose of 0.5 mg/kg bw. Animals of the vehicle control group were injected with the corresponding volume of saline. The open field test was used for the activity measurements 60 min after introduction of clonidine [7].

Haloperidol-induced catalepsy

For this experiment animals were randomly divided into 2 groups:
Group 1 - animals introduced with saline (10 mL/ kg bw, i.g.) before haloperidol injection (i.p.), n = 8.
Group 2 - animals treated with atristamine (100 mg/kg bw, i.g.) before haloperidol injection (i.p.), n = 8.
Catalepsy was induced with haloperidol (1 mg/kg bw, i.p.) and measured at 30-min intervals by means of the standard bar test [6]. This dose of haloperidol was chosen to induce a moderate degree of catalepsy, so that either attenuation or potentiation of the phenomenon could be detected. Atristamine in the dose of 100 mg/kg bw and saline for the control group were introduced i.g. 60 min before haloperidol i.p. injection.
At each time-point, both forepaws of the animal were gently imposed on the 3-mm-diameter wooden bar fixed horizontally 4 cm above the support. The length of time during which each animal maintained the initial position was measured. Mice were considered to be cataleptic if they maintained this position for 30 s or more. The end point of catalepsy was considered to occur when both forepaws were removed from the bar or if the animal moved its head in an exploratory manner. A cut-off time of 180 s was applied. Between time-point determinations the animals returned to their cages.

L-DOPA induced hyperactivity

For this experiment, animals were randomly assigned into 5 groups:
Group 1 - vehicle control group treated with saline (i.g.) one hour before saline i.p. injection, n = 8.
Group 2 - animals treated with atristamine (100 mg/kg bw, i.g.) one hour before saline i.p. injection, n = 8.
Group 3 - negative control group treated with 100 mg/kg bw of L-DOPA i.p. (too little dose for any manifestations of hyperactivity) one hour after saline i.g. introduction, n = 7.
Group 4 - animals treated with atristamine (100 mg/kg bw, i.g.) one hour before L-DOPA injection in the dose of 100 mg/kg bw, n = 8.
Group 5 - positive control group treated with 500 mg/kg bw of L-DOPA i.p. one hour after saline i.g. introduction, n = 8.
When mice were injected with L-DOPA stages of activity and aggressive behaviour were determined by a scoring system at every 15 min for 90 min after L-DOPA injection [25]. The different parameters of observation were piloerection, salivation, exophthalmos and hyperactivity. The scores were graded in the following manner: 0 - no effect; 1 - slight effect; 2 - moderate effect; 3 - marked effect. The total score in each time-point was calculated as a general indicator of the animal's state.

5-Hydroxytryptophan (5-HTP) induced head twitches

For this experiment, animals were randomly assigned into 5 groups:
Group 1 - animals treated with atristamine (100 mg/kg bw, i.g.) one hour before injection of saline (10 mL/kg bw, i.p.), n = 8.
Group 2 - negative control group treated with 50 mg/kg bw of 5-HTP (i.p.) one hour after saline i.g. administration, n = 8.
Group 3 - animals treated with atristamine (100 mg/kg bw, i.g.) one hour before 5-HTP injection in the dose of 50 mg/kg bw, n = 8.
Group 4 - positive control group treated with 300 mg/kg bw of 5-HTP (i.p.) one hour after saline i.g. administration, n = 7.
Group 5 - animals pre-treated with atristamine (100 mg/kg bw, i.g.) one hour before 5-HTP injection in the dose of 300 mg/kg bw, n = 8.
After 5-HTP or saline injection (group 1) each animal was placed in an individual cage, and the number of head twitches displayed by each mouse was counted. The number of head twitches for each animal within 1 minute was determined at the time-points 10, 20, 30, 40, 50 and 60 minutes after 5-HT precursor introduction. The time intervals between measurements for each animal were maintained [1].

Statistical analysis

The results are expressed as the mean (M) ± standard error of the mean (SEM). Statistical differences between groups were analysed using Student's t-test (in the case of normal distribution), the Mann-Whitney U test, and the Fisher angular transformation. The level of statistical significance was considered as $p < 0.05$.

Results and Discussion

Clonidine-induced aggressive behaviour

Clonidine, an antihypertensive drug, has been reported to possess marked sedative effects in human and animals and is a useful therapeutic agent for the treatment of agitated mental patients in very low doses [12]. However, clonidine in higher doses produces aggressive behaviour such as biting and attacking in mice housed in pair or groups and automutilation in mice housed individually in the absence of objects to bite [21]. Clonidine stimulates α2-adenoceptors in lower doses and αl-receptors in higher doses. Therefore, the pro-aggressive effect of clonidine (in high doses of the drug, e.g. 10 mg/kg bw) results from the stimulation of the postsynaptic α1-adrenergic receptor. However, clonidine-induced aggressive behaviour also has been reported to involve blockade of central adenosine receptors [5, 27].

It has been concluded before that atristamine alone given acutely or repeatedly in the dose of 100 mg/kg bw does not evoke any aggressive behaviour in mice.

As can be seen from Table I, a single dose (100 mg/kg bw, i.g.) of atristamine enhances the effect induced by clonidine (10 mg/kg bw), whereas a single dose of classical tricyclic antidepressant imipramine (25 mg/kg bw, i.p.) significantly decreases the number of attacks. Animals of the vehicle control group had several situational attacks. However, it cannot be attributed to the signs of aggressive behaviour.

Table I

The effect of single injections of atristamine and imipramine on the clonidine-induced aggressive behaviour in mice

Indicator	Group			
	Vehicle control group	Positive control group	Atristamine, 100 mg/kg bw, i.g.	Imipramine, 25 mg/kg bw, i.p.
Number of attacks	1.25 ± 0.75	30.3 ± 1.4	35.3 ± 1.3*/^	25.3 ± 0.9*
First attack latency, s	1020.0 ± 665.6	393.8 ± 33.9	305.0 ± 20.3^	455.0 ± 24.1

Notes. * - significant compared to the positive control group; ^ - significant compared to the imipramine group (Mann-Whitney U test).

It should be noted that the latency to the first attack in the group receiving atristamine reduced by 22.5% compared to the positive control group, whereas imipramine, conversely, prolonged this indicator by 15.5%.

It has been found that a number of typical and atypical antidepressants given chronically intensify clonidine-induced aggressiveness in mice. The data reported [9] supported further evidence for the hypothesis previously proposed that chronic, but not acute, administration of antidepressants enhances the responsiveness of central postsynaptic noradrenaline receptors. Antidepressants after acute administration exhibit contradictory effects depending on individual features.

Since the results of some experiments suggest that the clonidine-induced aggressive behaviour was not mediated through the alpha-2 adrenoreceptor, but rather the adenosine receptor, so the influence of atristamine on the adenosine receptors cannot be excluded. It correlates well with modulating influence of atristamine on the anxiogenic effect of caffeine in the open field test in mice [20].

Clonidine-induced depressive behaviour

Low doses of clonidine produce hypoactivity in mice and rats probably by stimulating pre-synaptic alpha 2-adrenoceptors in the brain [7]. The hypoactivity following clonidine administration has been proposed as a test model for antidepressants with a noticeable effect on the noradrenergic system.

It has been found that chronic pre-treatment with most of antidepressants results in a significant reduction in the clonidine-induced hypoactivity. However, results of the studies of the effect with acute administration of different antidepressants are highly ambiguous [2, 7].

The present findings indicate that clonidine in the dose of 0.5 mg/kg bw produced a pronounced hypoactivity in mice (Table II) in the open field test. It has been proven by extremely decreased ambulation, explorative activity and significantly prolonged latency in this group of animals. Pre-treatment with atristamine caused considerable, but not total, levelling of indicators in the open field test. The number of exploratory nose-pokes was the only indicator at the same level with the clonidine group.

Generally, clonidine (0.5 mg/kg bw) evoked a decrease in the total activity by 4.7 times ($p < 0.001$) compared to the vehicle group. Atristamine significantly reversed clonidine-induced depressive behaviour determined by an increase of the total

activity by 2.6 times ($p < 0.001$) compared to the clonidine group.

Table II

The effects of atristamine on clonidine-induced depressive behaviour of mice in the open field test

Indicator	Group, number of animals		
	Vehicle, n = 7	Clonidine, 0.5 mg/kg bw, n = 8	Clonidine, 0.5 mg/kg bw + atristamine, 100 mg/kg bw, n = 8
Latency, s	1.9 ± 0.3	5.9 ± 2.5*	1.5 ± 0.3^
I. Ambulation			
Square crossings	37.7 ± 2.5	11.4 ± 1.8***	34.3 ± 5.7^^^
II. Explorative activity			
Rearings	7.7 ± 1.8	0.9 ± 0.5**	5.1 ± 2.2^
Exploratory nose-pokes	34.0 ± 3.2	4.9 ± 1.2***	5.8 ± 0.9***
Total explorative activity	41.7 ± 2.1	5.8 ± 1.5***	10.9 ± 2.1***/^
III. Vegetative signs of emotional reactions			
Faecal boli	0.1 ± 0.1	0	0
Urinations	0.1 ± 0.1	0.1 ± 0.1	0.4 ± 0.3
Groomings	1.6 ± 0.7	0	0.1 0.1
Total number of emotional reactions	1.9 ± 0.9	0.1 ± 0.1	0.5 ± 0.3
The total activity			
I+ II + III	81.3 ± 2.4	17.3 ± 3.0***	45.6 ± 6.2**/^^^

Notes. *, **, *** - significant with $p < 0.05$, $p < 0.01$ and $p < 0.001$ compared to the vehicle control group; ^ and ^^^ - significant with $p < 0.05$ and $p < 0.001$ compared to the imipramine group (Mann-Whitney U test).

The above results indicate that acute administration of atristamine (100 mg/kg bw) increases the responsiveness of the adrenergic system (behavioural changes).

Haloperidol-induced catalepsy

The phenomenon of cataleptic immobility induced in rodents by typical neuroleptics (e.g., haloperidol, chlorpromazine, fluphenazine) is a robust behavioural method for studying the nigrostriatal function and its modulation by cholinergic, serotonergic, nitrergic, and other neurotransmitter systems [13].

The present study was designed to determine the acute effect of atristamine on haloperidol-induced catalepsy in mice using the standard bar test.

As can be seen from Table III, haloperidol produced a moderate catalepsy with the maximal intensity being observed at 60-120 min time points. Pre-treatment with atristamine led to attenuation of haloperidol-induced catalepsy in mice. It was clearly observed at first and final time-points by significant reduction of catalepsy duration by 54.0% ($p < 0.05$) and 64.7% ($p < 0.05$), respectively. At 60 - 150 min time-points atristamine also reduced the severity of catalepsy compared to control; it was determined by a short duration of catalepsy and decreased number of animals with total catalepsy. Thus, it may be concluded that atristamine causes certain activating effects on the dopaminergic neurotransmitter system attenuating the cataleptogenic effect of haloperidol.

Table III

The effects of atristamine on haloperidol-induced catalepsy in the bar test

Group of animals	Parameter	30 min	60 min	90 min	120 min	150 min	180 min
Saline + haloperidol, 1 mg/kg bw	Duration of catalepsy, s	107.0 ± 23.8	127.4 ± 21.2	139.0 ± 17.2	113.9 ± 20.6	99.6 ± 21.7	58.4 ± 17.9
	Animals with total catalepsy	3/8	4/8	4/8	3/8	2/8	0/8
Atristamine, 100 mg/kg bw + haloperidol, 1 mg/kg bw	Duration of catalepsy, s	48.8 ± 22.0*	97.8 ± 22.7	89.5 ± 20.6	67.9 ± 21.5	48.1 ± 18.4	20.6 ± 10.6*
	Animals with total catalepsy	1/8	2/8	2/8	1/8	0/8^	0/8

Notes. * - significant with $p < 0.05$ compared to the vehicle control group (Mann-Whitney U test); ^ - significant with $p < 0.05$ compared to the saline control group (Fisher angular transformation).

As well known, despite the pharmacological heterogeneity of selective serotonin reuptake inhibitors (SSRIs), they consistently attenuate neuroleptic-induced catalepsy [13]. However, non-

selective antidepressants (including tricyclic ones) have contradictory results when tested on the models of catalepsy caused by neuroleptics.

L-DOPA-induced hyperactivity

According to the data published a low dose of L-DOPA is insufficient to cause any visible signs of the hyperdopaminergic state, while a high dose (500 mg/kg bw) of the catecholamine precursor L-DOPA in animals produces autonomic and behavioural changes (piloerection, sweating, salivation, acceleration of respiratory rate, hyperactivity and irritability), which have been attributed to the newly formed products of its catabolic degradation (especially, dopamine) [8].

Table IV

The effects of atristamine on L-DOPA-induced hyperactivity in mice

Group	Piloerection	Hypersalivation	Exophthalmos	Hyperactivity	Score sum
15 min after L-DOPA injection					
Vehicle	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
Atristamine	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
L-DOPA 100	3.00 ± 0.0	0.0 ± 0.0	2.57 ± 0.20	1.00 ± 0.0	6.57 ± 0.20
L-DOPA 100 + atristamine	3.00 ± 0.0	0.25 ± 0.16^^^	2.87 ± 0.13	1.50 ± 0.33	7.63 ± 0.32*/^
L-DOPA 500	3.00 ± 0.0	1.75 ± 0.31	3.00 ± 0.0	1.50 ± 0.19	9.25 ± 0.49
30 min after L-DOPA injection					
Vehicle	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
atristamine	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
L-DOPA 100	2.85 ± 0.14	0.0 ± 0.0	2.14 ± 0.34	1.00 ± 0.0	6.00 ± 0.44
L-DOPA 100 + atristamine	2.50 ± 0.19	2.00 ± 0.38***	3.00 ± 0.0*	1.75 ± 0.31*	9.25 ± 0.49***
L-DOPA 500	2.88 ± 0.13	2.38 ± 0.26	2.88 ± 0.13	1.88 ± 0.13	10.0 ± 0.50
45 min after L-DOPA injection					
Vehicle	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
Atristamine	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
L-DOPA 100	2.28 ± 0.29	0.0 ± 0.0	1.57 ± 0.43	0.57 ± 0.20	4.42 ± 0.87
L-DOPA 100 + Atristamine	1.25 ± 0.31*/^^^	2.50 ± 0.27***	1.50 ± 0.33^^	1.38 ± 0.26*/^^	6.63 ± 0.73^^^
L-DOPA 500	3.00 ± 0.0	2.62 ± 0.18	2.75 ± 0.16	2.63 ± 0.18	11.0 ± 0.42
60 min after L-DOPA injection					
Vehicle	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
Atristamine	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
L-DOPA 100	0.86 ± 0.34	0.0 ± 0.0	0.43 ± 0.20	0.29 ± 0.18	1.57 ± 0.65
L-DOPA 100 + atristamine	0.13 ± 0.13^^^	1.63 ± 0.32***/^^	0.50 ± 0.19^^^	1.00 ± 0.0**/^^^	3.25 ± 0.50^^^
L-DOPA 500	2.38 ± 0.18	2.75 ± 0.16	2.88 ± 0.13	3.00 ± 0.0	11.0 ± 0.19
75 min after L-DOPA injection					
Vehicle	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
Atristamine	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
L-DOPA 100	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
L-DOPA 100 + atristamine	0.0 ± 0.0^^^	0.0 ± 0.0^^^	0.0 ± 0.0^^^	0.0 ± 0.0^^^	0.0 ± 0.0^^^
L-DOPA 500	1.50 ± 0.19	2.75 ± 0.16	2.25 ± 0.37	2.00 ± 0.33	8.50 ± 0.80
90 min after L-DOPA injection					
Vehicle	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
Atristamine	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
L-DOPA 100	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
L-DOPA 100 + atristamine	0.0 ± 0.0	0.0 ± 0.0^^^	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0^^^
L-DOPA 500	0.38 ± 0.18	1.63 ± 0.32	0.50 ± 0.27	0.50 ± 0.27	3.00 ± 0.66

Notes. *, **, *** -significant with $p < 0.05$, $p < 0.01$ and $p < 0.001$ compared to the negative control group; ^, ^^, ^^^ - significant with $p < 0.05$, $p < 0.01$ and $p < 0.001$ compared to the positive control group (Student's t-test).

As can be seen from Table IV and Figure 2, animals from the positive control group treated with 500 mg/kg bw L-DOPA had strongly pronounced signs of L-DOPA excitation (piloerection, hypersalivation, exophthalmos and hypercativity) for almost all observation times. Animals from the negative control group treated with the low dose of L-DOPA (100 mg/kg bw) had a pronounced piloerection and exophthalmos, but not hypersalivation and hyperactivity in the early time-points. After 45 min exposure the signs of L-DOPA administration started to decrease dramatically and in 60 min only insignificant symptoms were observed. Animals pre-treated with atristamine in early stages of the L-DOPA hyperactivity development had scores similar to the positive control group (500 mg/kg bw of L-DOPA) but in 30 min of exposure all visual signs started to decrease dramatically approaching to the results of the negative control group (100 mg/kg bw of L-DOPA).

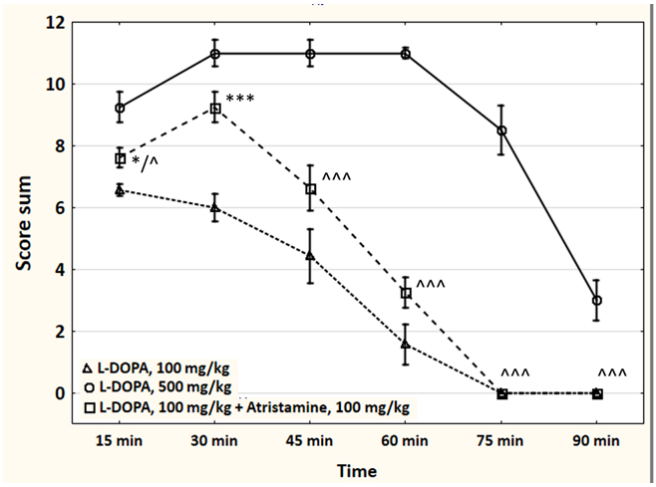

Figure 2.

The effects of atristamine on the L-DOPA-induced hyperactivity in mice

(*, *** - significant with $p < 0.05$ and $p < 0.001$ compared to the negative control group; ^ and ^^^ - significant with $p < 0.05$ and $p < 0.001$ compared to the positive control group (Student's t-test))

The present findings indicate that atristamine probably does not have MAO and COMT inhibition as the main mechanism of its antidepressant action. Otherwise, potentiation and prolongation of the L-DOPA induced hyperactivity after pre-treatment with atristamine in mice would be observed. Potentiation of L-DOPA (100 mg/kg bw) by atristamine in early stages of the hyperactivity development may be explained by the activating effect on the dopaminergic system; it has been also found in the bar test described above.

5-Hydroxytryptophan (5-HTP) induced head twitches in mice

Corne *et al*. described a simple method for quantitative and qualitative assessment of effects of drugs on behavioural responses produced by administration of 5-HTP. They showed that in parallel with the increase of the brain concentration of serotonin a particular head-twitch phenomenon occurred in mice, and the presence or frequency of this head-twitch could be taken as a sensitive measure of the agonistic or antagonistic effect of drugs [1]. The head-twitch response consists of a rapid and violent head shaking and does not occur in normal mice.

It was previously found that imipramine, desipramine, and amitriptylline inhibited the head-twitch response, whereas MAOI consistently potentiated it [1].

Analysis of the experimental data presented in Table V has proven that atristamine *per se* does not evoke the head-twitch response in mice. Animals treated with a low dose of 5-HTP (50 mg/kg bw) had an insignificant head-twitch response in mice that became almost indiscernible already 30 min after 5-HTP injection, whereas a high dose (300 mg/kg bw) of the pharmacological analyser produced a strongly pronounced head-twitch phenomenon in animals.

Table V

The effects of atristamine on head-twitch responses of mice

Group	n	Time-point				
		10 min	20 min	30 min	40 min	50 min
Atristamine, 100 mg/kg bw	8	0	0	0	0	0
5-HTP, 50 mg/kg bw	8	1.38 ± 0.42	1.50 ± 0.57	0.25 ± 0.16	0	0
5-HTP, 50 mg/kg bw + atristamine, 100 mg/kg bw	8	0.75 ± 0.49	0.75 ± 0.25	0.25 ± 0.16	0	0
5-HTP, 300 mg/kg bw	7	6.43 ± 1.66	8.43 ± 2.06	7.14 ± 2.27	5.14 ± 1.37	1.29 ± 0.47
5-HTP, 300 mg/kg bw + atristamine, 100 mg/kg bw	8	5.50 ± 1.05	13.38 ± 3.07	10.75 ± 2.79	3.88 ± 0.85	1.75 ± 0.49

Atristamine in the dose of 100 mg/kg bw introduced 1h before the 5-HTP introduction in both doses (50 and 300 mg/kg bw) had no significant effect on the head-twitch response in mice. However, the results of animals pre-treated with atristamine before the injection of 5-HTP low dose tended to be lower compared to the saline pre-treated group. And, *vice versa*, the results of animals received with atristamine and a high dose of 5-HTP prone to be higher than in the corresponding control group. Thus, in spite of the apparent absence of significant differences in

results they revealed complicated effects of atristamine on the 5-hydroxytryptamine neurotransmitter system even after a single dose. This influence can be described as modulating.

It was concluded that atristamine most likely had no effect on MAO since potentiation and prolongation of the 5-HTP induced head-twitch response in both doses after pre-treatment with the compound tested were not observed.

Conclusions

Interrelations between the effects of atristamine and serotonin, dopamine and norepinephrine neurotransmitter systems *in vivo* using the corresponding pharmacological analysers have been studied. The above results indicate that acute administration of atristamine (100 mg/kg bw) increases the responsiveness of the adrenergic system. It has been proven by the enhancement of clonidine-induced aggressive behaviour and the attenuation of clonidine-induced depression in mice. However, the influence of atristamine on the adenosine receptors cannot be excluded.

It has been shown that atristamine attenuates haloperidol-induced catalepsy in mice, but this effect does not associate with MAO and COMT inhibition. A mild modulating effect of atristamine on the serotonin neurotransmitter system was discovered using 5-HTP induced head-twitch response in mice. Consequences that could be produced by MAO inhibition also were not determined.

It has been concluded that the antidepressant action of atristamine may be explained predominantly by its complicated influence on the serotonin, dopamine and norepinephrine neurotransmitter systems. Certain correlations between results of atristamine and different antidepressants have been discussed.

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