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PRELIMINARY COMMUNICATION

INFLUENCE OF NEW γ-AMINOBUTYRIC ACID AMIDE DERIVATIVES AND ITS PHTHALIMIDE PRECURSORS ON THE CENTRAL NERVOUS SYSTEM ACTIVITY IN MICE

Anna Mendyk[1], Kinga Sałat[1], Tadeusz Librowski[1,#], Ryszard Czarnecki[1], Barbara Malawska[2]

[1]Department of Pharmacodynamics, [2]Department of Pharmaceutical Chemistry, Jagiellonian University, Medical College, Medyczna 9, PL 30-688 Kraków, Poland

Influence of new γ-aminobutyric acid amide derivatives and its phthalimide precursors on the central nervous system activity in mice. A. MENDYK, K. SAŁAT, T. LIBROWSKI, R. CZARNECKI, B. MALAWSKA. Pol. J. Pharmacol., 2001, 53, 689–693.

The present study investigated the influence of BM-78, BM-121 (γ-aminobutyric acid amide derivatives) and BM-42, BM-43 (phthalimide precursors of BM-78 and BM-121) on the spontaneous locomotor activity and on the picrotoxin-induced seizures. Results of pharmacological *in vivo* examination of the effects of new γ-aminobutyric acid amide derivatives and its phthalimide precursors (compounds BM-78, BM-121, BM-42, BM-43), presented in this paper showed that all the compounds had different but clear influence on CNS in mice.

***Key words**: GABA analogs, locomotor activity, picrotoxin-induced seizures, strychnine-induced seizures, lethality, mice*

INTRODUCTION

4-Aminobutyric acid (GABA) is the principal inhibitory neurotransmitter involved in the control of neuronal activity in the mammalian central nervous system (CSN) and in the regulation of many physiological mechanisms [1, 6]. It has been estimated that, depending on the brain area, GABA-ergic synapses make 20–50% of all synapses present in the CNS, and that the concentration of this acid is 200–1000 times as high as the concentration of other neurotransmitters [10, 11, 13]. A deficiency in GABA has been further implicated in epilepsy, schizophrenia and Huntington's and Parkinson's diseases. Several current hypotheses about the mechanisms of epilepsy suggest that enhancing the func-

correspondence; e-mail: mflibrow@kinga.cyf-kr.edu.pl

tion of inhibitory GABA neurons may be an important factor in controlling many seizures [1, 6, 7].

The mechanism of action of anticonvulsants includes potentiation of inhibitory mechanisms (predominantly the GABA system) by affecting the inhibitory synaptic processes, and inhibition of excitatory mechanisms (predominantly the glutamate system) and ionic channels by inhibiting excessive neuronal firing (modulation of membrane cation conductance *via* sodium, calcium or potassium channels) [1, 3, 4, 12]. GABA plays an important role in the etiology and control of epilepsy by mediating the inhibitory processes. Recent data have suggested that the inhibitory action of GABA mediated by low-affinity receptors could involve a regulation of the activity of voltage-gated calcium channels [10].

Better understanding of seizure pathophysiology led to the development of newer generation of antiepileptic drugs (AEDs) (felbamate, fosphenytoin, gabapentin, lamotrigine, oxcarbazepine, tiagabine, topiramate, vigabatrin, zonisamide). Gabapentin, tiagabine, vigabatrin act by potentiation of GABAergic transmission, as gabapentin increases brain GABA synthesis, tiagabine blocks the GAT-1 transporter and consequently inhibits synaptic GABA reuptake, while vigabatrin irreversibly inhibits GABA transaminase. Other new AEDs, whose main mechanism of action relates to the inhibition of sustained repetitive firing, through the blockade of voltage-dependent sodium channels and consequent inhibition of the release of excitatory neurotransmitters, also act by potention of GABAergic transmission (topiramate), by potentiation of postsynaptic GABA responses (felbamate), or by an increase in brain GABA and taurine levels (lamotrigine) [1, 4].

In search for the new anticonvulsants, we decided to examine the new γ-aminobutyric acid amide derivatives, i.e.: N-(4-fluorobenzylamide)-2-(4-phenylpiperazin-1-yl)-4-aminobutyric acid (BM-78), N-(4-methylbenzylamide)-2-(4-phenylpiperazin-1-yl)-4-aminobutyric acid (BM-121) and its phthalimide precursors, i.e.: N-(4-fluorobenzylamide) of 2-(4-phenylpiperazine)-4-phthalimidobutyric acid (BM-42), N-(4-methylbenzylamide) of 2-(4-phenylpiperazine)-4-phthalimidobutyric acid (BM-43), focusing on their pharmacological effects on CNS. Compounds BM-42 and BM-43 possess the phthalimide pharmacophore which has been reported to be applied for the fruitful design of several new and potent aniconvulsant compounds [2, 14].

Preliminary pharmacological tests on the synthesized compounds have been conducted in the framework of the Antiepileptic Drug Development (ADD) Program, Epilepsy Branch, Neurological Disorders Program, National Institutes of Neurological and Communicative Disorders and Stroke (NINCDS), in Bethesda, USA. Phase I. Studies of the investigated compounds involved three tests: maximal electroshock seizure (MES), subcutaneous pentetrazole (scMet) and neurologic toxity (Tox). All of the investigated compounds showed protection against MES and/or scMet seizures in mice [8, 9].

We attempted to investigate the influence of BM-78, BM-121, BM-42 and BM-43 on spontaneous locomotor activity and convulsions induced by picrotoxin. All tests were conducted in mice. Investigations of the effect of the compounds BM-42 and BM-43 on the spontaneous motor activity and picrotoxin-induced seizures were carried out 45 min and 3 h after *po* administration.

MATERIALS and METHODS

Animals

The studies were carried out on male Albino Swiss mice (18–24 g). The animals were kept in groups of 15 mice in type III-1290 cages (26.5 × 42.0 × 15.0 cm) at a room temperature of 22 ± 2°C, under 12/12 h light/dark cycle (light on from 7 a.m. to 7 p.m.), and had free access to food (standard laboratory pellets; Bacutil, Motycz, Poland) and water before the experiments. Each experimental group consisted of 6–12 animals/dose, and all the animals were used only once. The experiments were performed between 8 a.m. and 3 p.m.

Drugs

BM-78, BM-121, BM-42 and BM-43 (synthesized at the Department of Pharmaceutical Chemistry, Jagiellonian University, Medical College in Kraków) were suspended in 0.5% methylcellulose. Picrotoxin (Fluka Chemie AG), and strychnine were dissolved in natrium chloride (Rhone-Poulenc Rorer). Compounds BM-78, BM-121, BM-42 and BM-43 and were given *po*, while picrotoxin was administered *sc.* Control animals were given appropriate amounts of the vehicle.

Spontaneous locomotor activity

The spontaneous locomotor activity of individual mice was measured in photoresistor actometers (circular cages, 30 cm in diameter, provided with two photocells, and connected to the impulse counter), in 30-min sessions. BM-78, BM-121, BM-42, BM-43 were administered 45 min and BM-42, BM-43 3 h before the test.

Picrotoxin-induced convulsions

Groups of 6–8 mice were treated orally with the test compound in the form of suspension in 0.5% methylcellulose. Forty five minutes after oral administration of γ-aminobutyric acid amide derivatives (BM-78, BM-121) or 3 h after oral administration of its phthalimide precursors (BM-42, BM-43) the animals were injected *sc* with 3.2 mg/kg of picrotoxin. This dose produced convulsions in 100% of non-pretreated animals.

During the next 90 min, the animals were observed for the following symptoms: clonic seizures, tonic seizures, and death. Times of onset of seizures and time to death have been recorded.

Statistical analysis

U-Mann-Witney test (for analyzing the data concerning number of seizures) or Student's *t*-test (for analyzing the data concerning time of onset of seizures and the data from Table 1) were used to determine the statistical significance of differences between the control and treatment groups. Differences were considered significant when $p < 0.05$.

RESULTS and DISSCUSION

Investigations of the effects of the compounds BM-78, BM-121, BM-42 and BM-43 on the spontaneous motor activity in mice showed that, in comparison with the control group, the compounds BM-78 at a dose of 30 mg/kg and BM-121 at doses of 30 mg/kg and 300 mg/kg significantly increased locomotor activity in mice (Tab. 1). On the other hand, BM-121 at a dose of 100 mg/kg significantly decreased motor activity in animals. BM-42 was practically inactive 45 min after administration. BM-43 at all doses increased mice's spontaneous movement 45 min after the administration, but 3 h after drug administration at doses of 30 mg/kg and 100 mg/kg it decreased spontaneous locomotor activity in mice (Tab. 1).

Table 1. The influence of the investigated compounds on spontaneous locomotor activity

Compound	Dose (mg/kg)	Locomotor activity counts/30 min	
		Mean ± SEM	%
Control	–	301.6 ± 43.2	100
	30	485.9 ± 49.8**	161.1
BM-78	100	382.5 ± 28.8	126.8
	300	278.9 ± 55.2	92.4
Control	–	276.4 ± 25.8	100
	30	391.7 ± 37.3*	141.7
BM-121	100	123.0 ± 41.9***	44.5
	300	473.6 ± 66.8**	171.3
Control	–	134.6 ± 21.2	100
	30	135.0 ± 14.5	100.3
BM-42	100	141.7 ± 10.0	105.3
	300	135.1 ± 16.1	100.4
Control	–	503.1 ± 58.5	100
	30	466.3 ± 52.4	92.7
BM-42[1]	100	489.0 ± 47.1	97.2
	300	414.0 ± 71.0	82.3
Control	–	384.7 ± 37.9	100
	30	464.5 ± 50.4	120.7
BM-43	100	498.75 ± 48.27	129.6
	300	427.9 ± 49.1	111.2
Control	–	464.2 ± 72.4	100
	30	284.5 ± 27.4*	61.3
BM-43[1]	100	420.8 ± 81.5	90.7
	300	856.7 ± 108.4**	184.6

* $p < 0.05$, ** $p < 0.02$, *** $p < 0.01$. [1] When the compound was investigated 3 h after administration

Investigations of the effect of the compounds BM-78, BM-121, BM-43 and BM-42 on the picrotoxin-induced seizures in mice showed that, in comparison with the control groups, the compounds BM-121, BM-78 and BM-43 extended time of onset of convulsions. The last compound, BM-42, was practically inactive, but to some extent it affected number of seizures.

Compound BM-78 at doses of 30 mg/kg and 300 mg/kg reduced the number of seizures too. Compound BM-121 was practically inactive but at a dose of 30 mg/kg it insignificantly increased this parameter. Compound BM-43 had practically no influence on number of seizures. All these results are not statistically significant (Tab. 2).

Table 2. The influence of the investigated compounds on picrotoxin-induced convulsions in mice

Compound	Dose (mg/kg)	Time of onset of seizures (min)	Number of seizures
Control	–	12.0 ± 1.0	2.3 ± 0.4
	30	43.0 ± 15.0	0.7 ± 0.2**
BM-78	100	39.0 ± 12.8	1.7 ± 0.4
	300	43.8 ± 14.8	1.0 ± 0.4
Control	–	15.3 ± 1.0	1.7 ± 0.3
	30	27.3 ± 12.7	1.8 ± 0.8
BM-121	100	39.5 ± 15.9	1.7 ± 0.4
	300	27.7 ± 12.5	1.0 ± 0.2
Control	–	50.3 ±17.5	1.3 ± 0.8
	30	42.0 ± 15.6	1.3 ±0.5
BM-42	100	66.3 ± 15.0	0.5 ± 0.3
	300	69.0 ± 13.3	0.3 ± 0.2
Control	–	18.5 ± 4.7	2.0 ± 0.2
	30	30.2 ± 12.0	1.0 ± 0.3*
BM-42[1]	100	16.7 ± 1.5	1.3 ± 0.2
	300	23.2 ± 1.8	1.0 ± 0.0**
Control	–	43.0 ± 15.4	1.8 ± 0.9
	30	41.7 ± 15.3	1.0 ± 0.4
BM-43	100	16.2 ± 1.5	1.3 ± 0.2
	300	15.5 ± 1.7	1.3 ± 0.2
Control	–	11.2 ± 0.2	1.7 ± 0.2
	30	42.5 ± 15.1	1.0 ± 0.4
BM-43[1]	100	28.2 ± 12.4	1.5 ± 0.6
	300	29.3 ± 12.3	1.0 ± 0.36

* $p < 0.05$, ** $p < 0.02$. [1] When the compound was investigated 3 h after administration

Results of *in vivo* pharmacological examination of the effects of new γ-aminobutyric acid amide derivatives and its phthalimide precursors (compounds BM-78, BM-121, BM-42, BM-43), presented in this paper showed that all the compounds had different but clear influence on CNS in mice.

Compounds BM-42 and BM-43 (derivatives of γ-phthalimidobutyric acid) were more active in the tests conducted 3 h after their *po* administration. This confirms the results obtained in preliminary investigations in Bethesda. It is possible that the activity of BM-42 and BM-43 depends on their decomposition products such as derivatives of γ-aminobutyric acid [5, 6]. BM-42 showed high activity in picrotoxin-induced seizures. The lack of influence on locomotor activity in mice is consistent with its anticonvulsant activity [5].

In the test of picrotoxin-induced seizures the compound BM-78 was also active. It lengthened the time of onset of seizures and markedly decreased the number of seizures, particularly when given at a dose of 30 mg/kg. But its influence on the locomotor activity in mice was rather unclear and inconsistent with the anticonvulsant activity. The compound BM-121 had statistically significant, but ambiguous influence on the spontaneous locomotor activity in animals.

In summary, we can conclude that all obtained data are preliminary ones and we are still carrying out further experiments, which will help us to estimate the real value of the GABA analogues and their derivatives.

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