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3-Piperidynemethyl-5-chlorobenzoxazolinone-2 (PMB) Pharmacological Estimation of the Central Action

3-Piperydynometylo-5-chlorobenzoksazolinon-2 (PMB). Farmakologiczna ocena działania centralnego

3-пиперидин-метил-5-хлорбензохазолинон-2 (PMB). Фармакологическая оценка центрального действия

It has been found in many studies that benzoxazolinone-2 derivatives exert analgesic, hypnotic, anticonvulsive, antibacterial and antihelmintic action (17, 18). Similar results were observed in many recent experiments with 16 mostly new synthetized benzoxazolinone-2 derivatives (10).

The subject of this study are pharmacological experiments on 3-piperidinemethyl-5-chlorobenzoxazolinone-2 (PMB) and an attempt to determine the relationship between its pharmacological action and chemical structure, basing on the fact, that active hydrogen replaced by aminomethyl group in a molecule of a chemical compound can bring advantageous pharmacological effects — e.g. an increase of action or a change of its direction (15). Some kinds of these compounds, known as Mannich bases, are used in medicine: Rolitetracycline, Furaltadon, Xanturil, Morinamid or Morazon.

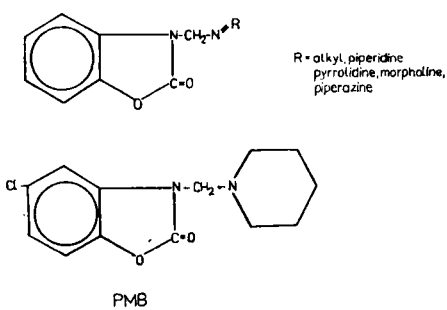

Fig. 1. Chemical structure of benzoxazolinone's-2 derivatives

In 3-aminomethyl derivatives of benzoxazolinone, as shown on Fig. 1, an atom of nitrogen can be saturated by alkyl groups or can be an element of cyclical structure such as pyrrolidine, piperidine, morpholine, piperazine (21).

MATERIALS AND METHODS

Albino Swiss mice of both sexes (16—28 g) and Wistar rats of both sexes (90—200 g) were injected peritoneally with the investigated compound suspended in a 3% aqueous solution of Tween 80. In most cases the substance was administered 15—30 min before tests. All results were analysed statistically using the Student t-test and experimental group consisted of 6—10 mice for rats. Acute toxicity — the mice were kept under observation for 48 hours and LD_{50} was determined according to the Litchfield and Wilcoxon (12) method. The research was limited to controling the influence of PMB on the central nervous system using the following tests:

1) walk on rotating rod according to Fog et al. (9) in mice;

2) spontaneous activity in mice by using photocell actometers;

3) effect on mice behavior in the hole-test determined by Boissier et al. (1);

4) effect on rectal temperature in mice by applying thermistor thermometer;

5) effect on electrogenic convulsion in mice according to Swinyard et al. (19);

6) interaction with amphetamine (2 mg/kg s.c.) by measuring hypermotility in mice;

7) interaction with caffeine (10 mg/kg i.p.) by measuring hypermotility in mice;

8) interaction with hexobarbital (65 mg/kg), chloral hydrate (260 mg/kg) and ethanol (50% — 4.5 g/kg) by measuring sleeping-time;

9) effect on the stereotyped behavior of mice and rats according to Braestrup (3);

10) effect on the haloperidol-induced catalepsy in mice (1 mg/kg i.p.) and in rats (2 mg/kg i.p.) according to Costall et al. (6);

11) effect on the spontaneous activity produced by reserpine (1.5 mg/kg s.c.), α-methyl-p-thyrosine (250 mg/kg i.p.), p-chlorophenylalanine (300 mg/kg i.p.), reserpine+α-methyl-p-thyrosine and diethyldithiocarbamate (200 mg/kg i.p.) in mice;

12) analgesic action in mice according to Nilsen (14) (the analgesic action was indicated by an increase in the intensity of current; the effect was registered as squeak of the mouse);

13) analgesic action in mice in hot-plate test (8).

Biochemical activity — the brain levels of 5-HT and 5-HIAA were determined spectrofluorimetrically according to the Curzon and Green (7) method in mice and rats. The turnover rate of 5-HT was calculated from the decline rate of 5-HIAA after inactivation of MAO by tranylcypromine (10 mg/kg i.p.) according to Tozer, Neff and Brodie (20). The brain concentration of NA and DA was estimated in mice with the method of Chang (5). The rate of NA and DA turnover was determined by the disappearance of NA and DA in the brain following a blockade of tyrosine hydroxylase with α-MT (300 mg/kg i.p.).

RESULTS

Acute toxicity. LD_{50} of PMB was 460 mg/kg (370—560). After administration of PMB in doses smaller than LD_{50}, abolition of righting reflex, perspiration and respiration distemper was observed. These effects disappeared in 3 hr.

Pharmacodynamic action. Significant stimulation was registered after PMB administration in doses 1/10, 1/20, 1/40 LD_{50} in locomotor activity and in interaction with caffeine (Table 1).

PMB in dose 1/10 LD_{50} acted significantly hypothermically decreasing temperature about 3.0°C and in dose 1/20 LD_{50} significantly hyperthermically increasing temperature to above 1.0°C in 60 min after its administration (Fig. 2).

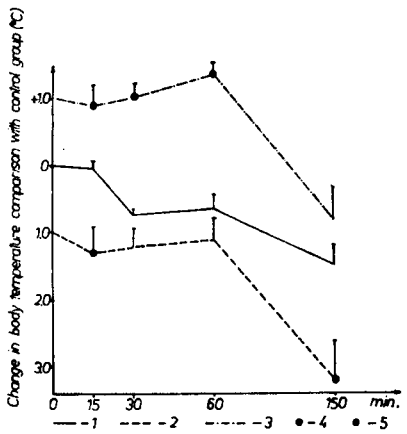

Fig. 2. The effect of various doses of PMB on body temperature in mice; 1 — controls, 2 — 1/10 LD_{50} PMB (46 mg/kg), 3 — 1/120 LD_{50} PMB (23 mg/kg), 4 — $p<0.05$, 5 — $p<0.001$

PMB administered in dose 1/10 LD_{50} completely reduced the hexobarbital, chloral hydrate and ethanol sleeping-time; the same effects were observed in interaction 1/20 LD_{50} of PMB and chloral hydrate and/or ethanol and 1/40 LD_{50} of PMB and ethanol (Table 2).

PMB in dose 1/10 LD_{50} only 120 min after injection enhanced amphetamine-induced stereotypy in mice and had no effect on this kind of stereotypy when applied in doses 1/15 and 1/10 LD_{50} in rats. On the other hand, PMB alone administered in doses 1/5 and 1/10 LD_{50} evoked stereotypy in mice and rats, persisting respectively for 120 min after injection and for 225 min (1/5 LD_{50}) to 300 min (1/10 LD_{50}), Fig. 3.

Doses 1/5 and 1/10 LD_{50} of PMB diminished haloperidol-induced catalepsy, especially in mice with maximal effects in 15—300 min (1/10 LD_{50}) and in 45—300 min (1/5 LD_{50}) after injection (Fig. 4).

Tab. 1. The influence of PMB on spontaneous, caffeine

Drugs /mg/kg/ i.p.	Spontaneous activity after time /min/ /$\bar{x}$ ±SE/				Caffeine
	30	60	90	120	30
Tween 80	100.0 ± 9.8	100.0 ± 28.7	100.0 ±28.1	100.0 ± 37.3	100 ± 5.01
PMB /46.0/	296.0 ±53.6**	363.5 ±112.8*	294.0 ±79.9*	236.5 ± 54.3*	402 ± 52.8**
PMB /23.0/	284.6 ±34.1**	165.5 ± 43.0	124.3 ±32.5	428.6 ±167.8	210 ± 11.9**
PMB /11.5/	229.8 ±41.1**	417.4 ± 77**	233.1 ±63.5	266.7 ± 80.8	174 ± 29.3*
PMB /5.75/	114.1 ±25.4	109.6 ± 37.7	75.0 ±29.7	128.6 ± 49.2	82 ± 21.7

* $p<0.05$; ** $p<0.001$.

Tab. 2. The influence of PMB on drug — induced narcosis in mice

Drug /mg/kg/ i.p.	Sleeping time /min ±SE/		
	Hexobarbital /65 mg/kg/	Chloral hydrate /260 mg/kg/	50% Ethanol /4.5 g/kg/
Tween 80	23 ±2.26	53 ±4.67	30 ±4.04
PMB /46/	no sleep	no sleep	no sleep
PMB /23/	25 ±7.45	no sleep	no sleep
Tween 80	12 ±1.32	-	-
PMB /11.5/	15 ±1.87	-	no sleep
Tween 80	-	-	13 ±1.60
PMB /5.7/	-	-	19 ±2.30*

* $p<0.05$.

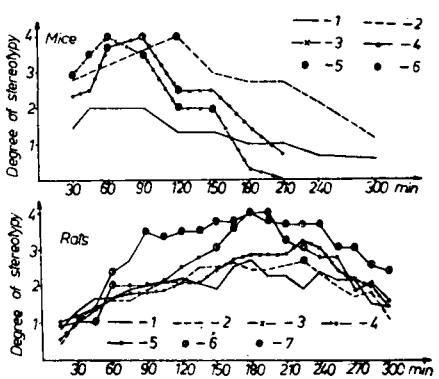

Fig. 3. The influence of PMB on the amphetamine-induced stereotypy and application of PMB as a drug induced stereotypy in mice and rats. Mice: 1 — amphetamine 5 mg/kg i.p., 2 — amphetamine+1/10 LD_{50} PMB, 3 — 1/10 LD_{50} PMB, 4 — 1/5 LD_{50} PMB, 5 — $p<0.05$, 6 — $p<0.001$. Rats: 1 — amphetamine 5 mg/kg i.p., 2 — amphetamine+1/15 LD_{50} PMB, 3 — amphetamine+1/10 LD_{50} PMB, 4 — 1/10 LD_{50} PMB, 5 — 1/5 LD_{50} PMB, 6 — $p<0.05$, 7 — $p<0.001$

and amphetamine activity in mice as percent of control

induced hyperactivity after time /min/ /$\bar{x}$ ±SE/			Amphetamine activity after time /min/ /$\bar{x}$ ±SE/			
60	90	120	30	60	90	120
100 ± 9.25	100 ± 17.6	100 ± 24.0	100 ±25.2	100 ±20.3	100 ±15.8	100 ±14.3
609 ±111.7***	687 ±112.1***	698 ±100.7***	190 ±52.1	185 ±42.7	178 ±42.5	178 ±42.5
221 ± 56.6*	122 ± 28.7	103 ± 25.8	-	-	-	-
287 ± 62.6**	272 ± 75.4**	287 ± 81.6**	-	-	-	-
69 ± 23.3	80 ± 27.8	59 ± 20.3	-	-	-	-

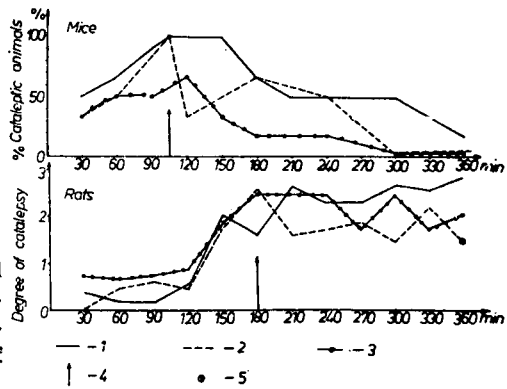

Fig. 4. The influence of PMB on haloperidol-induced catalepsy in mice and rats; 1 — haloperidol 1 mg/kg, 2 — haloperidol+1/10 LD_{50} PMB, 3 — haloperidol+1/5 LD_{50} PMB, 4 — time of application of PMB, 5 — $p<0.05$

PMB in dose 1/10 LD_{50} reversed reserpine (60 and 90 min after injection), α-MT (30—90 min), PCPA (60 and 90 min), reserpine+α-MT (30—90 min) and DDC actions (30—90 min) after PMB administration (Table 3).

PMB in dose 1/10 LD_{50} exerted analgesic activity determined by enhanced intensity of current necessary to produce pain reaction in 30—120 min after its injection in electric test and shortened the time of appearance of pain reaction in 120 min after injection in dose 1/10 LD_{50} in thermical test (Table 4).

Biochemical activity. PMB increased 5-HT and DA concentration and decreased NA concentration in mice, had no effect on the turnover rate and time, but increased DA turnover rate in mice. PMB given to rats increased slightly 5-HIAA concentration, 5-HT turnover rate and decreased slightly 5-HT turnover time in rats (Tables 5, 6 and 7).

Tab. 3. The influence of reserpine, α-MT, PCPA, reserpine+α-MT and DDC on the locomotor activity induced by PMB in mice

Drug /mg/kg/ i.p., s.c.	Spontaneous activity after time /min/ /$\bar{x}$ ±SE/		
	30	60	90
Tween 80	124.00 ± 8.98	123.00 ±23.50	30.00 ±72.79
PMB /46.0/	216.00 ±66.60	763.00 ±257.40	718.00 ±171.60
Reserpine /1.5/	2.00 ± 1.00	1.60 ± 1.25	5.00 ± 2.67
Reserpine + PMB	66.00 ±26.91	240.00 ±115.28*	617.00 ±192.00**
Tween 80	107.00 ±20.95	92.00 ±22.21	53.00 ±18.43
PMB /46.0/	354.00 ±67.61	589.00 ±113.90	580.00 ±155.78
α-MT /250.0/	86.00 ±11.55	22.00 ± 6.14	16.00 ± 5.32
α-MT + PMB	355.00 ±96.70*	520.00 ±96.68**	561.00 ±114.04**
Tween 80	107.00 ±20.95	92.00 ±22.21	53.00 ±18.43
PMB /46.0/	354.00 ±67.61	589.00 ±113.90	580.00 ±155.78
PCPA /300.0/	89.00 ± 7.99	94.00 ±18.27	102.00 ± 4.79
PCPA + PMB	276.00 ±36.25	688.00 ±32.14**	898.00 ±83.49**
Tween 80	127.00 ±12.60	158.00 ±27.78	84.00 ±32.60
PMB /46.0/	403.00 ±42.60	669.00 ±76.20	457.00 ±81.29
Reserpine +α-MT	2.00 ± 1.43	2.60 ± 1.78	2.30 ± 2.33
Reserpine +α-MT + PMB	43.00 ±10.40**	109.00 ±24.00**	74.00 ±14.32**
Tween 80	95.00 ±30.26	95.00 ±28.95	94.00 ±41.60
PMB /46.0/	263.00 ±70.70	439.00 ±91.27	109.00 ±30.93
DDC /200.0/	69.00 ±24.50	36.00 ±20.50	6.00 ± 1.35
DDC + PMB	278.00 -76.92*	351.00 -102.63**	325.00 -108.31**

* $p<0.05$; ** $p<0.001$; reserpine was injected 18 hr before PMB, α-MT 1 hr before PMB, PCPA in turn by 3 days before the test, DDC 2 hr. before PMB.

Tab. 4. The influence of PMB on the pain reaction caused by electric and thermic stimulus in mice

Drug /mg/kg/ i.p.	Intensity of current /μA/ evoking the pain reaction after time /min/ /$\bar{x}$ ±SE/				Appearance time of the pain reaction /sec/ after time /min/ /$\bar{x}$ ±SE/			
	30	60	90	120	15	30	60	120
Tween 80	7.5 ±0.13	7.1 ±0.15	7.1 ±0.31	7.2 ±0.27	9.0 ±0.91	10.0 ±0.86	10.0 ±0.87	11.0 ±1.00
PMB /46/	17.7 ±0.78**	15.6 ±0.89**	9.7 ±0.74*	9.7 ±0.55**	7.0 ±0.99	9.0 ±1.02	9.0 ±1.39	7.0 ±0.88**

* $p<0.05$; ** $p<0.001$.

Tab. 5. The influence of PMB on the NA, DA, 5-HT and 5-HIAA concentration on the whole brain of the mice and rats

Drugs /mg/kg/ i.p.	Species of animal	Brain contents /% of control ±SE/			
		NA	DA	5-HT	5-HIAA
Tween 80	rats	—	—	100.0 ±7.47	100.0 ±5.31
PMB /46/	rats	—	—	94.9 ±7.97	107.5 ±4.38
Tween 80	mice	100.0 ±2.60	100.0 ±3.30	100.0 ±3.33	100.0 ±9.42
PMB /46/	mice	78.2 ±1.89**	114.1 ±1.50**	140.0 ±2.83**	96.4 ±6.57

** $p<0.001$; animals were killed 1 hr after injection.

Tab. 6. The influence of PMB on the NA and DA concentration in the whole brain of mice, after treatment with α-MT (300 mg/kg)

Drug /mg/kg/ i.p.	Brain contents /µg/g ±SE/	
	NA	DA
Tween 80	0.551 ±0.019	1.170 ±0.015
PMB /46.0/	0.415 ±0.019 *	1.320 ±0.067*
α-MT /300.0/	0.316 ±0.016	0.530 ±0.028
α-MT + PMB	0.272 ±0.019*	0.425 ±0.031*

* $p<0.05$; α-MT was injected 2 hr before PMB, mice were killed 1 hr after PMB.

Tab. 7. The influence of tranylcypromine (10 mg/kg) on the brain turnover rate of 5-HT reduced after PMB in rats and mice

Drug /mg/kg/ i.p.	Species of animal	Brain level of 5-HIAA before MAO blockade /µg/g ±SE/	Rate constant of 5-HIAA loss after IMAO K /hr^{-1} ±SE/	Turnover rate of 5-HT /µg/g/hr/	5-HT turnover time /min/
Tween 80	rats	1.073 ±0.057	0.755 ±0.10	0.75	79.4
PMB /46/	rats	1.154 ±0.047	0.798 ±0.20	0.85	75.2
Tween 80	mice	1.2856 ±0.070	0.656 ±0.049	0.77	91.36
PMB /46/	mice	1.2897 ±0.099	0.658 ±0.063	0.78	91.18

PMB was injected 1 hr before tranylcypromine; animals were killed 45 and 90 minutes after tranylcypromine.

DISCUSSION

Our experimental compound — PMB attracts attention especially because of its resemblance to amphetamine and its derivatives, but what is particularly interesting is its capacity to induce stereotypy. This effect seems to confirm the stimulating action of PMB on the central nervous system (11). Because some PMB properties differ from those of amphetamine, it could be rather classified as a member of the methylphenidate group of amphetamine-like stimulants by the antagonism of its behavioral effects with α-MT (4). Moreover, it appeared that PMB increases DA brain concentration. This finding seems to be comparable with methylphenidate group, which selectively increases brain DOPAC and it may be secondary to an increase in DA synthesis. The methylphenidate group derivatives caused a long-lasting increase not only in DOPAC but also in HVA, in contrast to amphetamine. The results confirm that stereotypy-inducing amphetamine-like drugs can be classified into at least two groups according to whether they are inhibited or not by a high dose of reserpine and by the reverse sensitivity to inhibition by synthesis inhibitor-α-MT. These results are believed to indicate that PMB as a methylphenidate-group member releases DA from granular stores, while amphetamine releases new synthetized DA (2). The PMB receptor's strong affinity (dopaminergic affinity) may be supported by its high degree of stereotypy-induced behavior, such as licking, gnawing and biting. Although a modulatory, but unspecified role of NA on dopaminergically mediated behavior was suggested by Randrup (16), Mogilnicka and Braestrup (13) noted that an increase in NA transmission inhibits the extreme features of stereotyped behavior. The conclusion of the present study implies that stereotyped behavior elicited by PMB is dependent on DA transmission but its mechanism of action may be also influenced by central NA transmission (its interaction with not only α-MT but α-MT+reserpine and DDC).

The specific action of PMB as a central stimulant may be explained by the presence of piperidine substituent in the molecule of benzoxazolinone, unobserved in its most approximated structural analogues such as morpholine, piperazine and pyrrolidine derivatives (10). The fact that some psychotonic, antidepressant and analeptic drugs as well as some antagonist of narcotic drugs have also a piperidine ring (coupled with amphetamine molecule) in their structure may be some confirmation of the above hypothesis. Such kinds of drugs are: Pipradrol and Methylphenidate, Phacetoperane and anorexigenic Diphemethoxidine. Another kind of drugs are those in which the oxazole system is included in amphetamine structure and this structure may be responsible for their central

stimulating effects (psychoanaleptic Pemoline, antidepressant Tozalinon, anorexigenic Clominorex).

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STRESZCZENIE

Przeprowadzono badania ośrodkowego działania nowo zsyntetyzowanego związku 3-piperydynometylo-5-chlorobenzoksazolinonu-2 (PMB). Stwierdzono, że związek wywiera dzielanie pobudzające, związane ze stymulają neuronów dopaminergicznych. Opierając się na tym można by sugerować, iż uzyskany efekt jest związany z obecnością pierścienia piperydynowego w pozycji 3 PMB.

РЕЗЮМЕ

Проведено исследования центрального действия новосинтетизированного соединения 3-пиперидин-метил-5-хлорбензоксазолинон-2 (PMB). Доказано, что это соединение обладает возбуждающим действием связанным со стимуляцией допаминергических невронов. На фоне этого можно предполагать, что этот эффект связан с присутствием пиперидинового кольца в позиции 3 PMB.