ANNALES
UNIVERSITATIS MARIAE CURIE-SKŁODOWSKA
LUBLIN — POLONIA
VOL. XLIII, 23 SECTIO D 1988

Katedra i Zakład Farmakologii. Akademia Medyczna w Lublinie
Kierownik: prof. dr hab. Zdzisław Kleinrok

Artur WAMIL, Zdzisław KLEINROK

Role of Noradrenergic System in Limbic Seizures Induced by Pilocarpine. I. Importance of β-adrenergic Receptor

Rola układu noradrenergicznego w drgawkach limbicznych wywołanych pilokarpiną. I. Znaczenie receptora β-adrenergicznego

Роль адренергической системы в лимбических судорогах вызваных пилокарпином. I. Значение β-адренергического рецептора

1. INTRODUCTION

The neurophysiological and pharmacological studies emphasize that the limbic system of the brain shows the proclivity to develop seizures (11, 24). The intrahippocampal and intraamygdaloid injection of cholinomimeticts and intraperitoneal injection of pilocarpine in rats produce brain damage which conforms closely to that observed in nervous tissue in autopsied brains of epileptic patients (13, 41). Systemic administration of pilocarpine in rats produces, with the exception of neuropathological changes, a number of phenomena including preconvulsive behaviour, severe limbic seizures, generalized clonic-tonic phase, status epilepticus and spontaneous recurrent seizures even a few months after single injection of pilocarpine. This experimental model of epilepsy has been found optimal to show the effects of drugs on the development of pathological activity within the limbic circuits and efficacy of antiepileptic drugs and other agents as a preventive and therapeutic approches to temporal lobe epilepsy (34, 39).

Among the neurotransmitters studied to date, serotonin (19), GABA (43), dopamine (42), purines (40), excitatory amino acids (30) and peptides (44) provide protection in this model. Futhermore, it has been known for some time that central noradrenergic neurons and the noradrenergic tracts play an important role in the seizure susceptibility (10, 28). The influence of noradrenergic transmission on seizures was recently studies in models of acute generalized (27), chronic generalized (18) and partial chronic epilepsies (9, 12). In generalized

seizures induced by external factors, e.g., toxic or metabolic disorders, in normal brain, noradrenaline may act as an endogenous anticonvulsant (10). In partial chronic epilepsy induced by kindling or cobalt, the noradrenergic stimulation is less involved in the onset of the focal epileptic syndrome than in the restriction of its spread and generalization (8, 12).

Microiontophoretic studies of noradrenaline and noradrenergic receptors agonists and antagonists in the mammalian cortex suggest that β-adrenergic receptor stimulation induces inhibitory responses (33, 38). Inhibitory influence of *locus coeruleus* neurons on target cells in the cortex is mediated by β-adrenoreceptors, too (10). In contrast, in the dentate gyrus of isolated hippocompal slices, noradrenaline exhibits a β-receptor mediated prolongation of epileptiform discharges evoked by high-frequency stimulaton and lowering extracellular Mg^+ concentration (37). Taking into consideration the concept that β_1- and β_2-subtype-adrenoreceptor can be independently regulated (29), their effects upon epileptic activity may be different.

We would like to complete the above evidence, studying the influence of noradrenergic receptor agonist and antagonist drugs, and substances which increase or decrease the level of endogenous norepinephrine in the brain upon the partial acute epilepsy induced by a potent cholinergic agonist pilocarpine. The role of β-adrenergic receptor seems to be the most important part of our investigations.

2. MATERIALS AND METHODS

2.1. General

Experiments were carried out on adult, male Wistar rats weighing 160–250 g, housed under standard laboratory conditions (natural light-dark cycle), rodent chow pellets (Murigran, Bacutil, Poland) and tap water provided *ad libitum*. The experimental groups consisted of 6 to 10 randomly assigned rats.

2.2. Convulsive procedure

The animals were injected intraperitoneally (i.p.) with pilocarpine in doses of 200, 300, 400 mg/kg. After injections each rat was subsequently put individually into plastic compartment (40 × 25 × 17 cm) and during 180 min the behavioural changes were recorded. The observation period for the occurrence of the clinic phase of limbic seizure activity was 120 min and the mortality of rats was also noted in the two periods of time: 0–30 min; 31–180 min since pilocarpine administration. The animals not convulsing were considered to possess 120 min latency time. Scopolamine methyl nitrate, not penetrating the blood-brain barrier substance was injected subcutaneously (s.c.) in the dose of 1 mg/kg 30 min prior to the all injections of pilocarpine in order to minimize its peripheral cholinergic effects (4). The convulsive tests were performed in a quiet room with a average temperature of 22 $\pm$2°C between 2:00–7:00 p.m. Control groups were injected with respective vehicles and challenged using the same solution of pilocarpine and scopolamine methyl nitrate.

2.3. Score of behavioural alterations

Appreciation of the behavioural changes after pilocarpine administration in rats was estimated in the scoring system according to the observed stages (Table 1).

2.4. Drugs

Pilocarpine hydrochloride (Merck, Darmstadt) and scopolamine methyl nitrate (Sigma, St. Louis, MO) were freshly dissolved in distilled water immediatelly before use. The following drugs affecting β-adrenergic receptor were used: isoprenaline sulfate, propranolol hydrochloride (Polfa, Warszawa), salbutamol sulfate (Polfa, Poznań), pindolol (Visken; Sandoz, Nurnberg). Additionally: lidocaine hydrochloride (Xylocain; Astra Lakemedel, Sodertalje), 6-hydroxydopamine hydrobromide, pargyline hydrochloride (Sigma, St. Louis, MO). Isoprenaline, licocaine, pargyline, pilocarpine, propranolol and scopolamine were sufficiently soluble in distilled water. Pindolol and salbutamol were suspended in a 3% solution of Tween 81 (Loba Chemie, Wien). 6-Hydroxydopamine (6-OHDA) was dissolved in 0.9% saline and 0.2% solution of ascorbic acid (Polfa, Kraków) was also added.

All the drugs and agents were administered i.p. except scopolamine methyl nitrate, s.c. and 6-OHDA intracerebroventricularly (i.c.v.). Pilocarpine, pindolol and salbutamol were injected in a volume of 5 ml/kg, isoprenaline, lidocaine, pargyline, propranolol and scopolamine methyl nitrate were injected in a volume of 2.5 ml/kg. 6-OHDA was injected in a volume of 4 µl/rat.

2.5. Injection of 6-OHDA i.c.v.

In order to obtain the selective depletion of norepinephrine level in the whole brain for about 50% (16) the rats under aether anaesthesia received injections of 6-OHDA into the right cerebral ventricle. The following coordinates were used to position the microneedle of Hamilton microsyringe: AP +1; L 1.5; V 3.2 (15).

Fifty µg of 6-OHDA (6-OHDA HBr, weight expressed as free base) dissolved in 4 µl of 0.9% saline containing 0.2 mg/ml ascorbic acid as antioxidant, were infused at the rate of 2 µl/min. To permit diffusion of the drug the microneedle was left in place for and additional minute after the end of the injection. Thirty min before 6-OHDA, pargyline was injected in the dose of 50 mg/kg i.p. (17). The rats were used two weeks after injections of 6-OHDA or vehicle. Vehicle-injected animals served as controls.

2.6. Evaluation of the results and the statistical analysis

The ED_{50} values for motor limbic seizures induced by pilocarpine were calculated according to the graphical method of Litchfield and Wilcoxon (23).

In treated and control groups the scoring system of seizure parameters (0 to 6 points) was compared by means of Mann-Whitney *U* test and latency to the onset of seizures was evaluated by means of Student *t*-test.

3. RESULTS

3.1. Pilocarpine-induced seizures

Behavioural alterations produced by pilocarpine i.p. in studied doses were time dependent. After pilocarpine (200 mg/kg) extremely preconvulsive beha-

viour was observed (stage: 0, 1 or 2) (Table 1). The mean ±SE was 0.83 ±0.13 points. After pilocarpine administration in doses of 300 and 400 mg/kg normal behaviour (stage 0) was not observed. All the animals showed preconvulsive and convulsive activity (stage: 1 to 4) and a few of them died (stage: 3 + 1; 3 + 2; 4 + 1; 4 + 2). Using the dosage of 300 mg/kg of pilocarpine mean ±SE was 2.25 ±0.16 points and latency to onset of seizures was 72.5 ±21.3 min. After pilocarpine (400 mg/kg) the mean ±SE was 4.16 ±0.11 points and the time to onset of seizures was 37.1 ±16.7 min. The sequence of all the stages of

Table 1. Seizure score in rats following i.p. injection of pilocarpine in doses of 200, 300 and 400 mg/kg

Score (x)	Stage
0	normal behaviour
1	akinesia and staring, gustatory and olfactory automatisms, body tremor
2	nodding and/or wet dog shakes
3	limbic forelimb clonic seizures with rearing
4	tonic-clonic seizures, failing and/or status epilepticus

x + 2 death of animal 30 min, after pilocarpine administration;
x + 1 death between 31 and 180 min.

the scoring system was presented in rats treated with pilocarpine in the dose of 400 mg/kg i.p. The preconvulsive behaviour started almost immediately after injection. All the animals became motionless, crouched on all limbs and stared. After 5 to 10 min the phase of olfactory and gustatory automatisms predominated in the form of mouth movements with prominent chewing, mild salivation, teeth chattering. At the same time there was observed tremor of the whole body (stage 1). After the next few minutes nodding and/or wet dog shakes were observed (stage 2). This activity lasted from 12 to 40 min after injection and developed into the next phase (stage 3), motor limbic seizures (forelimbs clonus with rearing and falling) and intense salivation. When this activity was extended, the tonic-clonic seizures and death were observed or limbic status epilepticus with salivation and bloodied tears, even for a few hours.

Out of 24 rats injected with pilocarpine 200 mg/kg i.p., 17 exhibited extremely preconvulsive activity.

Out of 85 animals injected with pilocarpine 300 mg/kg, 39 had clonic seizures,

Table 2. Behavioural changes observed following the i.p. injection of pilocarpine
Values are number of rats showing behavioural abnormalities/number of rats used

Pilocarpine (mg/kg i.p.)	Score							Score: 0 to 6 points (mean ±SE)
	0	1	2	3	4	5	6	
200	7/24	14/24	3/24	–	–	–	–	0.83 ±0.13
300	–	43/85	2/85	13/85	24/85	1/85	2/85	2.25 ±0.16
400	–	18/161	–	14/161	74/161	16/161	39/161	4.16 ±0.11

8 exhibited the tonic phase, 25 limbic status epilepticus and 7 died within the 180 min observation period.

Among the 161 rats injected with pilocarpine 400 mg/kg, 143 showed clonic phase, 48 had the tonic seizures, 97 limbic status epilepticus and 47 died within the period of observation (Table 2).

3.2. Effects of drug affecting β-adrenergic receptor upon pilocarpine-induced seizures (Table 3)

Among the agonists studied, isoprenaline in the dose of 2 mg/kg injected 10 min before pilocarpine (300 mg/kg) completely blocked appearance of seizures (0.66 ±0.33 points), and latency was 120 ±0 min, the maximal assumed. The

Table 3. Influence of drugs affecting β-adrenergic receptors upon limbic seizures induced by pilocarpine (300 and 400 mg/kg i.p.)

Treatment (mg/kg i.p.)	Treatment time (min)	Time of onset (min ±SE) max. 120 min	Score: 0 to 6 points (mean ±SE)
Control (300)	–	72.5 ±21.3	2.25 ±0.16
Isoprenaline (0.2)	10	85.1 ±16.6	3.0 ±0.81
Isoprenaline (0.5)	10	99.3 ±9.8	2.16 ±0.4
Isoprenaline (2)	10	120.0 ±0.0[a]	0.66 ±0.33[a]
Isoprenaline (0.2)	120	38.8 ±16.4	3.66 ±0.7
Isoprenalina (0.5)	120	44.8 ±15.4	3.16 ±0.3
Isoprenaline (2)	120	48.2 ±16.2	3.5 ±0.6
Salbutamol (1)	15	59.6 ±14.1	3.4 ±0.6
Salbutamol (2.5)	15	64.0 ±15.3	3.1 ±0.64
Salbutamol (5)	15	9.4 ±1.7[b]	4.2 ±0.32[b]
Salbutamol (7.5)	15	32.0 ±9.8	4.2 ±0.4[b]
Salbutamol (10)	15	55.9 ±14.6	3.8 ±0.63[a]
Propranolol (0.5)	60	107.8 ±12.1	1.16 ±0.6
Propranolol (2)	60	120.0 ±0.0[a]	0.0 ±0.0[b]
Propranolol (5)	60	106.3 ±13.6	1.66 ±0.49
Propranolol (15)	60	61.2 ±17.5	2.5 ±0.66
Pindolol (0.1)	45	78.4 ±15.2	1.66 ±0.55
Pindolol (0.5)	45	76.2 ±14.6	1.6 ±0.33
Pindolol (1)	45	96.1 ±12.3	1.4 ±0.48
Pindolol (4)	45	36.8 ±20.4	3.2 ±0.79
Control (400)	–	37.1 ±16.7	4.16 ±0.11
Propranolol (0.5)	60	48.1 ±12.5	3.5 ±0.54
Propranolol (1)	60	57.5 ±17.0	2.9 ±0.72
Propranolol (2)	60	94.8 ±13.4[a]	1.4 ±0.58[c]
Propranolol (3)	60	72.6 ±15.8	2.2 ±0.55[b]
Propranolol (5)	60	60.4 ±16.3	2.8 ±0.49[a]
Propranolol (7.5)	60	44.6 ±13.1	4.6 ±0.41
Propranolol (15)	60	14.1 ±2.1	4.8 ±0.49

Explanation: [a] – $p < 0.05$; [b] – $p < 0.01$; [c] – $p < 0.001$ versus control group (Student t-test and Mann-Whitney U test).

lower doses of isoprenaline and the longer time between isoprenaline and pilocarpine injections were ineffective on the pilocarpine-induced seizures. On the other hand, salbutamol (5, 7.5, 10 mg/kg) intensified the action of pilocarpine in the dose of 300 mg/kg, respectivelly, 4.2 ±0.4 points; 3.8 ±0.63 points. Additionally, salbutamol in dose of 5 mg/kg shortened the time to onset to 9.4 ±1.7 min.

Antagonist of β-adrenergic receptor, propranolol in the dose of 2 mg/kg before pilocarpine (300 mg/kg) completely abolished the enhanced action of pilocarpine and the animals presented normal behaviour (0 ±0 points). The higher doses of propranolol were ineffective in its anticonvulsant action. The other β-blocker, pindolol in the dose 1 mg/kg showed a weak anticonvulsant effect, which was not observed when the drug was administered in lower and higher doses. Furthermore, proconvulsive action of pilocarpine in the dose of 400 mg/kg was distinctly blocked by propranolol in the dose of 2 mg/kg (1.4 ±0.58 points; 94.8 ±13.4 min), 3 mg/kg 2.2 ±0.55 points and 5 mg/kg (2.8 ±0.49 points). The anticonvulsant effect of pindolol against pilocarpine in this dose was not observed.

3.3. Effect of combined action of lidocaine and propranolol upon pilocarpine-induced seizure activity (Table 4)

Lidocaine injected in the dose of 100 mg/kg before pilocarpine (400 mg/kg) significantly blocked convulsions (0.83 ±0.48 points) and prolonged the time to onset of seizures (108.8 ±11.17 min). The combined action of lidocaine in

Table 4. Effect of combined treatment of lidocalne and propranolol upon limbic seizures induced by pilocarpine (400 mg/kg i.p.)

Treatment (mg/kg i.p.)	Treatment time (min)	Time of onset (min ±SE) max. 120 min	Score: 0 to 6 points (mean ±SE)
Control (400)	–	37.1 ±16.7	4.16 ±0.11
Lidocaine (20)	75	42.0 ±15.8	3.5 ±0.67
Lidocaine (50)	75	39.8 ±16.2	3.33 ±0.49
Lidocaine (100)	75	108.8 ±11.17[b]	0.83 ±0.48[c]
Propranolol (2)	60	94.8 ±13.4[a]	1.4 ±0.58[c]
Propranolol (10)	60	44.6 ±13.1	4.6 ±0.41
Propranolol (15)	60	14.1 ±2.1	4.8 ±0.49
Lidocaine (20) +	75		
+ Propranolol (2)	60	120.0 ±0.0[c]	0.2 ±0.13[c]
Lidocaine (20) +	75		
+ Propranolol (10)	60	82.5 ±12.3	1.1 ±0.43[c]
Lidocaine (20) +	75		
+ Propranolol (15)	60	96.8 ±12.7	2.1 ±0.38[c]

Explanation: [a] – $p < 0.05$; [b] – $p < 0.01$; [c] – $p < 0.001$ versus control group (Student t-test and Mann-Whitney U test).

the dose of 20 mg/kg and propranolol in the dose of 2, 10 and 15 mg/kg resulted in a protection against seizure activity although lidocaine 20 mg/kg or propranolol (10 and 15 mg/kg) alone were ineffective in this model of seizures.

3.4. Effect of combined action of propranolol and salbutamol upon pilocarpine-induced seizures (Table 5)

Injection of pilocarpine after the combined administration of propranolol and salbutamol in doses inhibiting or potentiating seizures, respectively, resulted in the decrease of the proconvulsive activity of salbutamol, especially when propranolol was given in the dose of 2 mg/kg.

Table 5. Effect of combined treatment of propranolol and salbutamol upon seizures induced by pilocarpine (300 and 400 mg/kg i.p.)

Treatment (mg/kg i.p.)	Treatment time (min)	Time of onset (min ±SE) max. 120 min	Score: 0 to 6 points (mean ±SE)
Control (300)	–	72.5 ±21.3	2.25 ±0.16
Propranolol (0.5)	60	107.8 ±12.1	1.16 ±0.6
Propranolol (2)	60	120.0 ±0.0[a]	0.0 ±0.0[b]
Propranolol (5)	60	106.3 ±13.6	1.66 ±0.49
Salbutamol (5)	15	9.4 ±1.7[b]	4.2 ±0.32[b]
Propranolol (0.5) +	60		
+ Salbutamol (5)	15	32.6 ±17.7	3.5 ±0.5
Propranolol (2) +	60		
+ Salbutamol (5)	15	83.5 ±23.1	2.16 ±1.22
Propranolol (5) +	60		
+ Salbutamol (5)	15	37.8 ±17.1	4.0 ±0.52
Control (400)	–	37.1 ±16.7	4.16 ±0.11
Propranolol (2)	60	94.8 ±13.4[a]	1.4 ±0.58[c]
Propranolol (5)	60	60.4 ±16.3	2.8 ±0.49[a]
Salbutamol (5)	15	16.2 ±2.69	5.66 ±0.21[a]
Propranolol (2) +	60		
+ Salbutamol (5)	15	51.0 ±21.8	3.66 ±0.84
Propranolol (5) +	60		
+ Salbutamol (5)	15	16.2 ±3.33	4.16 ±0.4

Explanation: [a] – $p < 0.05$; [b] – $p < 0.01$; [c] – $p < 0.001$ versus control group (Student *t*-test and Mann-Whitney *U* test).

3.5. Effect of propranolol or salbutamol upon pilocarpine-induced seizures in rats treated with 6-hydroxydopamine (Table 6)

A partial selective decrease of noradrenaline level in rat brain produced significant potentiation of seizures, when pilocarpine was injected in the dose of 400 mg/kg. The convulsive activity of pilocarpine (300 mg/kg) was not altered. Salbutamol (5 mg/kg) increased the seizure activity in rats with lesions of

noradrenergic presynaptic membranes induced by 6-OHDA to the similar extent when compared with unlesioned rats. Propranolol (2 mg/kg) significantly prolonged the latency to seizures in 6-OHDA-pretreated animals but did not affect the seizure score.

Table 6. Effect of salbutamol (5 mg/kg i.p., 15 min prior pilocarpine) or propranolol (2 mg/kg i.p., 60 min prior pilocarpine) upon limbic seizures induced by pilocarpine (300 and 400 mg/kg i.p.) in rats treated previously with 6-hydroxydopamine (50 μg/rat i.c.v.)

Treatment (mg/kg i.p.)	Time of onset (min ±SE) max. 120 min	Score: 0 to 6 points (mean ±SE)
Control (300)	72.5 ±21.3	2.25 ±0.16
Salbutamol	9.4 ±1.7[b]	4.2 ±0.32[b]
6-OHDA	82.0 ±15.52	2.7 ±0.67
6-OHDA + Salbutamol	19.2 ±2.6[bf]	4.8 ±0.32[cd]
Control (400)	37.1 ±16.7	4.16 ±0.11
Propranolol	94.8 ±13.4[a]	1.4 ±0.58[c]
6-OHDA	18.6 ±1.34	5.6 ±0.27[b]
6-OHDA + Propranolol	43.1 ±10.8[d]	4.8 ±0.34

Explanation: [a] – $p < 0.05$; [b] – $p < 0.01$; [c] – $p < 0.001$ versus pilocarpine-control, [d] – $p < 0.05$; [e] – $p < 0.01$; [f] – $p < 0.001$ versus 6-OHDA + pilocarpine control (Student *t*-test and Mann-Whitney *U* test).

4. DISCUSSION

The potent convulsive effect of pilocarpine observed in the present study seems mediated by stimulation of the central cholinergic receptors (41, 42). The excitatory action of acetylocholine probably works through a direct postsynaptic action via muscarinic and nicotinic receptors (5, 35) and presynaptic disinhibition (6, 20). Doses of pilocarpine used in experiments extremely affect this neurotransmission. Probably these two mechanisms can be responsible for seizures induced by pilocarpine.

The present results show that noradrenergic modification of seizures in this model is dependent on the subtype of β-adrenergic receptor stimulation or blockade. It was shown that norepinephrine hyperpolarized neurons in various regions of the brain and spinal cord (32). This effect was antagonized by β-adrenergic blockers and by inhibitors of the (Na^+, K^+ — ATP-ase (33). Thus, protective action of noradrenaline against seizures can be mediated via β-adrenoceptors. However, the blockade of β-adrenoceptors reduce seizures in this model so the effects mediated by the two β-receptors subtypes cannot be the same yet.

In amygdaloid kindled rats a selective β-adrenoceptor down regulation was shown whereas other receptor populations were unchanged (3). In this context a connection between seizure activity and β-adrenoceptors may be postulated.

β_1- and β_2-adrenergic agonist, isoprenaline in the dose of 2 mg/kg administered 10 min before pilocarpine significantly inhibited seizure activity. Salbutamol, β_2-agonist potentiated the convulsive response. This may lead to the conclusion that stimulation of β_1-adrenoceptors can induce antiepileptic effects. The decrease in seizure activity was observed after injection of propranolol in smaller doses (2 to 5 mg/kg) but not in doses of 7.5 to 15 mg/kg. Other non-selective β-adrenergic antagonist, pindolol (1 mg/kg) weakly protected animals against seizures but was ineffective in the dose of 4 mg/kg. Interestingly, the investigation of tremor which also appears as a preconvulsive behaviour after pilocarpine administration induced by harmaline aminooxidase inhibitor demonstrated that blockade of β_2-adrenoceptors by butoxamine selective β_2-antagonist inhibited tremor and isoprenaline in the dose of 0.1 mg/kg but not acebutalol selective β_1-adrenergic antagonist decreased the effect of butoxamine (31).

To show a membrane stabilizatory effect of propranolol the combined treatment with lidocaine was studied. In the dose of 100 mg/kg lidocaine was a very potent anticonvulsant. The combined treatment with ineffective dose of lidocaine (20 mg/kg) and propranolol in the protective dose (2 mg/kg) decreased the seizure score. The combination of lidocaine (20 mg/kg) and propranolol in ineffective doses (10 and 15 mg/kg) showed a decrease of seizure activity, too. It could be suggested that anticonvulsant activity of β-blockers is connected both with β-adrenergic receptor and membrane stabilization.

In order to confirm proconvulsive activity of salbutamol via β-receptor, salbutamol (5 mg/kg) and propranolol (0.5 to 5 mg/kg) were injected. Propranolol in the dose responsible for the highest anticonvulsant activity (2 mg/kg) completely reversed the effect of salbutamol in the most proconvulsive dose (5 mg/kg).

On the basis of these findings it could be suggested that stimulation of β_1-adrenoceptors or blockade of β_2-adrenoceptors provide anticonvulsant effects and stimulation of β_2-adrenoceptors or blockade of β_1-adrenoceptors increase seizure susceptibility.

These results are in good agreement with data indicating role of β-receptor in seizures induced by pentylenetetrazol (25). In these experiments propranolol administered orally produced significant anticonvulsant effect which depended on the enantiomer used. (−)-Propranolol was seven times more effective than the (+)-enantiomer. These results indicated that both mechanisms: β-adrenoceptor blockade and membrane stabilization played a role in the anticonvulsant effect of propranolol. Pindolol in those studies, a β-adrenergic antagonist which lacks membrane stabilizing activity (36) also possessed marked anticonvulsant activity. Propranolol was studied previously in a variety of epilepsy providing some protection against aminophylline-induced seizures (14), maximal electroshock (26), hyperbaric oxygen exposure (22) and loud auditory stimulation (2).

Moreover, cyanopindolol which blocks both β-adrenergic and 5-$HT_{1\beta}$ receptors strongly protected against pilocarpine-induced seizures in rats (19). In lower doses it showed anticonvulsant activity via receptor antagonism and in higher doses local anestetic component predominated. Timolol has much less membrane stabilizing and partial agonist activity, but protected against seizures, so its effect was probably mediated by central β-adrenoceptors. Selective β_1-antagonist practolol i.c.v. was ineffective, but selective β_2-antagonist ICI 118.551 was a powerful anticonvulsant (25).

Partial decrease of norepinephrine in the brain selectively changed the action of propranolol and salbutamol. Propranolol (2 mg/kg) proved ineffective to reduce seizures and only prolonged the latency to the onset of seizure versus 6-OHDA-control. Salbutamol (5 mg/kg) increased seizure activity and shortened the time to the onset of seizures both versus 6-OHDA-control and pilocarpine--control. The investigations of other autors showed that the animals treated with 6-OHDA exhibited increased seizure susceptibility depending on reduced norepinephrine but not dopamine level in the brain in pentylenetetrazol induced seizures (27, 28) kindled seizures (12) and audiogenic seizures (7). Ionophoretically administered norepinephrine to the cortex decreased epileptic activity via β-adrenoceptor in a chronic cobalt focus (38). Failure of anticonvulsant effect of propranolol injection in rats after subtotal destruction of central noradrenergic neurons, in this study, and enhanced effect of salbutamol can be associated with the up-regulation of noradrenergic receptors (21) and degeneration of presynaptic membranes (1).

In conclusion, this study provides evidence that in normal rats stimulation of β_1- or blockade of β_2-adrenoceptors is related to anticonvulsant action whereas stimulation of β_2- or blockade of β_1-adrenoceptors increases seizure intensity.

*

This work was supported by the Polish Science Research Fund, CPBP 06-02.I.9.

REFERENCES

1. Acheson A. L., Zigmond M. J.: Short and Long Term Changes in Tyrosine Hydroxylase Activity in Rat Brain after Subtotal Destruction of Central Noradrenergic Neurons. J. Neurosci. **1**, 493, 1981.
2. Anlezark G., Horton R., Meldrum B. S.: The Anticonvulsant Action of the (−)- and (+)-enantiomers of Propranolol. J. Pharm. Pharmacol. **31**, 482, 1979.
3. Ashton D., Leysen J. E., Wauquier A.: Neurotransmitters and Receptor Binding in Amygdaloid Kindled Rats: Serotonergic and Noradrenergic Modulatory Effects. Life Sci. **27**, 1547, 1980.
4. Baez L. A., Eakridge N. K., Schein R.: Postnatal Development of Dopaminergic and Cholinergic Catalepsy in the Rat. European J. Pharmacol. **36**, 155, 1976.
5. Benardo L. S., Prince D. A.: Cholinergic Pharmacology of Mammalian Hippocampal Pyramidal Cells. Neuroscience **7**, 1703, 1982.

6. Ben-Ari Y., Krnjević K., Reinhardt W., Ropert N.: Intracellular Observations on Disinhibitory Action of Acetylocholine in Hippocampus. Neuroscience **6,** 2475, 1981.
7. Bourn W. M., Chin L., Picchioni L.: Effect of Neonatal 6-hydroxydopamine Treatment on Audiogenic Seizures. Life Sci. **21,** 701, 1977.
8. Bregman S., Dedek J., Nasaif S., Trottier S., Chauvel P.: Noradrenergic Mechanisms Control Propagation of Chronic Cobalt Activities in the Rat. [in:] Cerebral Blood Flow, Metabolism and Epilepsy, eds. M. Baldy-Moulinier, D. H. Ingvar and B. S. Meldrum, John Libbey Eurotext, London–Paris 1983, p. 351.
9. Callaghan D. A., Schwark W. S.: Involvement of Catecholamines in Kindled Amygdaloid Convulsions in the Rats. Neuropharmacology **18,** 541, 1979.
10. Chauvel P., Trottier S.: Role of Noradrenergic Ascandling System in Extinction of Epileptic Phenomena, [in:] Advances in Neurology, eds. A. V. Delgado-Escueta, A. A. Ward Jr., D. M. Woodbury and R. J. Porter. Raven Press, New York 1986, p. 475.
11. Collins R. C., Tearse R. G., Lothman E. W.: Functional Anatomy of Limbic Seizures: Focal Discharges from Medial Entorhinal Cortex in Rat. Brain Res. **280,** 25, 1983.
12. Corcoran M. E., Mason S. T.: Role of the Forebrain Catecholamines in Amygdaloid Kindling. Brain Res. **190,** 473, 1980.
13. Corsellins J. A. N., Meldrum B. S.: Epilepsy, [in:] Greenfield's Neuropathology, eds. W. Blackwood and J. A. N. Corsellins, Arnold, London 1976, p. 771.
14. Czuczwar S. J., Janusz W., Wamil A., Kleinrok Z.: Inhibition of Aminophylline-induced Convulsions in Mice by Antiepileptic Drugs and Other Agents. European J. Pharmacol. **144,** 309, 1987.
15. Fifkova E., Marsala J.: Stereotaxic Atlases for the Cat, Rabbit and Rat, [in:] Electrophysiological Methods in Biological Research, eds. J. Bures, P. Mojmir and J. Zachar, Academic Press, New York–London 1967, p. 653.
16. Hadreen J. C., Chalmers J. P.: Neuronal Degeneration in Rat Brain Induced by 6-hydroxydopamine, a Histological and Biochemical Study. Brain Res. **47,** 1, 1972.
17. Jagiełło-Wójtowicz W.: Mechanisms of Central Action of Octopamine. Pol. J. Pharmacol. Pharm. **31,** 509, 1979.
18. Jobe P. C., Picchioni A. L., Chin L.: Role of Brain Norepinephrine in Audiogenic Seizures in the Rat. J. Pharmacol. Exp. Ther. **184,** 1, 1973.
19. Kleinrok Z., Janusz W.: The Role of Central Serotonergic Structures in Pilocarpine-induced Seizures in Rats, [in:] Abstracts of the First Polish-Swedish Symposium on Structure and Function in Neuropharmacology, Warsaw 1988, p. 34, 44.
20. Krnjevic K., Relffenstein R. J., Ropert N.: Disinhibitory Action of Acetylocholine in the Rats Hippocampus: Extracellular Observations. Neuroscience **6,** 2465, 1981.
21. Langer S. Z., Hicks P. E.: α-adrenergic Receptor Subtypes: Pharmacological and Therapeutic Implications, [in:] Adrenergic Receptors: Molecular Properties and Therapeutic Implications, eds. R. J. Lefkowitz and E. Lindenlaub, F. K. Schattauer Verlag, Stuttagart–New York 1985, p. 591.
22. Levy A., Nagi S. H., Finck A. D., Kawashima K., Spector S.: Disposition of Propranolol Isomers in Mice. European J. Pharmacol. **40,** 93, 1976.
23. Litchfield J. T., Wilcoxon F.: A Simplified Method of Evaluating Dose-effect Experiments. J. Pharmacol. Exp. Ther. **96,** 99, 1949.
24. Lothman E. W., Collins R. C.: Kainic Acid Induced Limbic Seizures: Metabolic, Behavioural, Electroencephalographic and Neuropathological Correlates. Brain Res. **218,** 299, 1981.
25. Louis W. J., Papanicolaou J., Summers R. J., Vajda F. J. E.: Role of Central β-adrenoceptors in the Control of Pentylenctetrazol-induced Convulsions in Rats. Br. J. Pharmacol., **75,** 441, 1982.
26. Madan B. R., Barar F. S. K.: Anticonvulsant Activity of Some β-adrenoceptor Blocking Agents in Mice. European J. Pharmacol. **29,** 1, 1974.

27. Mason S. T., Corcoran M. E.: Forebrain Noradrenaline and Metrazol-induced Seizures. Life Sci. **23,** 167, 1978.
28. Mason S. T., Corcoran M. E.: Catecholamines and Convulsions. Brain Res. **170,** 497, 1979.
29. O'Donnel R. S., Wanstall J. C.: Functional Evidence for Differential Regulation of β-adrenoceptor Subtypes. Trends Pharmacol. Sci. **8,** 265, 1987.
30. Patel S., Millan M.-H., Mello L. M., Meldrum B. S.: 2-Amino-7-phosphonoheptanoic acid (2-APH) Infusion into Entopeduncular Nucleus Protects against Limbic Seizures in Rats. Neurosci. Lett. **64,** 226, 1986.
31. Paul V.: Involvement of β_2-adrenoceptor Blockade and 5-hydroxytryptamine Mechanisms in Inhibition of Harmaline-induced Tremor in Rats. European J. Pharmacol. **122,** 111, 1986.
32. Phillis J. W.: The Role of Cyclic Nucleotides in the CNS. Can. J. Neurol. Sci. **4,** 151, 1977.
33. Phillis J. W., Wu P. H., Thierry D. L.: The Effect of α-, β-adrenergic Receptor Agonist and Antagonists on the Efflux of ^{22}Na and Uptake of ^{42}K by Rat Brain Cortical Slices. Brain Res. **236,** 133, 1982.
34. Rodin E. A.: The Prognosis of Patients with Epilepsy. (Thomas, Springfield, IL) 1982, p. 5.
35. Ropert N., Krnajevic K.: Pharmacological Characteristic of Facilitation of Hippocampal Population Spikes by Cholinomimetics. Neuroscience **7,** 1963, 1982.
36. Scriabine A.: β-adrenoceptor Blocking Drugs in Hypertension. A Rev. Pharmacol. Toxicol. **19,** 269, 1979.
37. Stanton P. K., Jones R. S. G., Mody I., Heinemann U.: Epileptiform Activity Induced by Lowering Extracellular (Mg^{2+}) in Combined Hippocampal-enthorinal Cortex Slices: Modulation by Receptors for Norepinephrine and N-methyl-D-aspartate. Epilepsy Res. **1,** 53, 1987.
38. Szabadi E.: Adrenoceptors on Central Neurons: Microelectrophoretic Studies. Neuropharmacology **18,** 831, 1979.
39. Turski W. A., Cavalheiro E. A., Coimbra C., da Penha Berzaghi M., Ikonomidou-Turski C., Turski L.: Only Certain Antiepileptic Drugs Prevent Seizures Induced by Pilocarpine. Brain Res. Revs. **12,** 281, 1987.
40. Turski W. A., Cavalheiro E. A., Ikonomidou C., Morales Mello L. E. A., Bortolotto Z. A., Turski L.: Effects of Aminophylline and 2-chloroadenosine on Seizures Produced by Pilocarpine in Rats: Morphological and Electroencephalographic Correlates. Brain Res. **361,** 309, 1985.
41. Turski W. A., Cavalheiro E. A., Schwarz M., Czuczwar S. J., Kleinrok Z., Turski L.: Limbic Seizures Produced by Pilocarpine in Rats: Behavioural, Electroencephalographic and Neuropathological Study. Behav. Brain Res. **9,** 315, 1983.
42. Turski L., Cavalheiro E. A., Turski W. A., Meldrum B. S.: Role of a Striatonigral Pathway in Regulation of the Threshold for Limbic Seizures in Rat, [in:] Advances in Epileptology, eds. P. Wolf, M. Dam, D. Janz, F. E. Dreifuss, Raven Press, New York 1987, p. 115.
43. Turski L., Schwarz M., Cavalheiro E. A., Turski W. A., Ikonomidou C., Sontag K. H.: Nigral GABA-ergic Mechanisms Modify the Development of Seizures Produced by Pilocarpine in Rats: Behavioural, Electroencephalographic and Neuroanatomical Evidence. J. Cerebral Blood Flow and Metabolism **5,** S363, 1985.
44. Wamil A., Croiset G., Kleinrok Z., De Wied D.: Beneficial Effect of ACTH 4-10 on Pilocarpine-induced Seizures. Neurosci. Res. Com. 1989 (in press).

Otrzymano 1988.12.28.

STRESZCZENIE

W badaniach przeprowadzonych na szczurach wykazano, że drgawki limbiczne wywołane pilokarpiną hamują stymulację receptorów adrenergicznych β_1 lub blokadę receptorów adrenergicznych β_2. Przeciwne efekty, tj. działanie prodrgawkowe, obserwuje się po stymulacji receptorów β_2 lub blokadzie receptorów β_1.

РЕЗЮМЕ

В исследованиях проведеных на крысах показано, что лимбические судороги вызванные пилокарпином тормозят стимулацию адренергических рецепторов β_1 или блокаду адренергических рецепторов β_2. Противоречевые эффекты т.е. просудорожное действие наблюдаеться после стимулации рецепторов β_2 или блокады рецепторов β_1.

ANNALES UNIVERSITATIS MARIAE CURIE-SKŁODOWSKA

Nakład 600 egz. + 25 nadb. aut., ark. wyd. 17, ark. druk. 14 + 48 str. wkl. kred. Papier druk. sat. III kl., B1, 70 g.
Oddano do składu w maju 1989 r., podpisano do druku w maju 1990 r., wydrukowano w czerwcu 1990 r.
Skład, łamanie i diapozytywy: Spółka z o.o. „VERSUS" w Lublinie, ul. Złota 2

Druk i oprawa: Lub. Zakł. Graf., Lublin, ul. Unicka 4, Zam. 975/90 Cena zł 10.200,—

ANNALES
UNIVERSITATIS MARIAE CURIE-SKŁODOWSKA
LUBLIN — POLONIA
VOL. XLII SECTIO D 1987

12. I. Urban, W. Stążka, I. Tychowska, S. Dyba: Participation of Histamine in the Cardiovascular Reactions in Response to ACTH.
Udział histaminy w reakcjach układu krążenia po podaniu ACTH.

13. G. Rzeszowska, E. Mazurek, I. Mierzejewska, M. Fijałka-Rymar, A. Ignaciuk: Wybrane wskaźniki hepatitis B, obecne w surowicy krwi w pierwszych dniach hospitalizacji u chorych na ostre wirusowe zapalenie wątroby (wzw).
Selected Indices of Hepatitis B, Present in Blood Serum on the First Days of Hospitalization in Patients with Viral Hepatitis (wzw).

14. G. Rzeszowska, E. Mazurek, I. Mierzejewska, M. Fijałka-Rymar, A. Ignaciuk: Miana wybranych wskaźników hepatitis B w surowicy krwi chorych w przebiegu ostrego wirusowego zapalenia wątroby (wzw).
Titres of Selected Indices of Hepatitis B in Blood Serum in the Course of Acute Viral Hepatitis (wzw).

15. S. Bryc: Biopsja cienkoigłowa sterowana ultrasonograficznie.
The Thin-needle Biopsy Guided by Ultrasound.

16. H. Gieryng, R. Gieryng: Zmiany histologiczne w mózgu myszy wywołane doświadczalnym zarażeniem pełzakami z grupy *limax*.
Histologic Changes in Brain of Mice Caused by Experimental Infection with Amoebas of the *limax* Group.

17. A. Wójcik, Z. Brzeski, Z. Borzęcki, A. Kot: Narażenie pracowników galwanizerni na niektóre metale ciężkie i cyjanki w procesie produkcji.
Exposition of a Galvanizing Plant's Workers to Some Heavy Metals and Cyanides in the Manufacturing Process.

18. J. Kudejko, B. Nestorowicz, G. Kądziela-Wypyska: Zmiany paznokciowe i uszne w przebiegu epilepsji u 6-letniego dziecka.
Onychodystrophy and Auricular Deformation in 6-year-old Child.

19. G. Orlicz-Szczęsna: Wpływ doświadczalnie wywołanych wrzodów żołądka u szczura na odczyny histochemiczne w błonie śluzowej dwunastnicy.
The Effect of Experimentally Induced Gastric Ulcers on Histochemical Reactions in the Duodenum Mucosa of the Rat.

20. A. Zarębska: Wpływ gonadektomii i podawania Mesteronu na komórki części zewnątrzwydzielniczej trzustki szczura białego.
The Influence of Gonadectomy and Mesterone Treatment on Pancreas Exocrine Cells of White Rats.

21. E. A. Jędrzejewska: Topochemical Examinations of the Lungs of the Rat after Combined Administration of Serpasil and Deslanatoside. I. Histochemical Examinations.
Badania topochemiczne płuc szczura po skojarzonym podawaniu Serpasilu i Deslanatosidu. I. Badania histochemiczne.

22. E. A. Jędrzejewska: Topochemical Examinations of the Lungs of the Rat after Combined Administration of Serpasil and Deslanatoside. I. Morphological Examinations.
Badania topochemiczne płuc szczura po skojarzonym podawaniu Serpasilu i Deslanatosidu. I. Badania morfologiczne.

23. R. Czajka, M. Łopucka, H. Źrubek, M. Semczuk, D. Kruk, W. Reszko, A. Czekierdowski: Ocena kardiotokograficzna monitorowania płodu w ciąży i porodzie w materiale Kliniki Położnictwa w Lublinie w latach 1976—1986.
Fetal Cardiotocographic Assessment During Pregnancy and Labour in the Material of Obstetrical Clinic in Lublin in the Years 1976—1986.

ANNA
UNIVERSITATIS MARIAE
LUBLIN —
VOL. XLII SECTI

24. G. Orlicz-Szczęsna: Zmiany morfologiczne
śluzowej żołądka szczurów przewlekl
Morphological Changes and Mono
Rats Under Chronic Stress Conditi

25. S. Bryc, J. Woźnica: The CT Assessment of Intrasellar Microadenomas.
Tomografia komputerowa w ocenie śródsiodłowych mikrogruczolaków.

26. T. Jastrzębski, K. Michałowski, M. Jazienicki: Problematyka kliniczna postrzałów śrutowych.
Clinical Problems with the Shotgun Wounds.

27. D. Misiuna, L. Bielak, S. Biliński, M. Kiełczykowska: Tautomeria układu 2-hydrazyno-4-fenylo-tiazol⇌hydrazon-4-fenylo-tiazol-2-onu. Pochodne acylowe. I. 4-fenylo-3-R-tiazol-2-ilideno- oraz β-metylo-β-(4-fenylo-tiazol-2-ilo)-hydrazydy kwasu octowego i benzoesowego.
The Tautomerism of 2-hydrazino-4-phenyl-tiazole⇌4-phenyl-thiazol-2-one hydrazone. The Derivatives of Acids. I. 4-phenyl-3-R-thiazol-2-ylidene and β-methyl-β-(4-phenyl-thiazol-2-yl)-hydrazides of Acetic and Benzoic Acid.

28. W. Stążka, R. Czabak, J. Kądziołka, W. Pawluczuk: Wpływ podania apomorfiny do komór mózgu na efekty depresyjne nerwu błędnego.
Effect of Intracerebroventricular Injection of Apomorphine on the Depressor Responses of Vagus Nerve.

29. J. Woźnica, S. Bryc, J. Olney: Wartość badania TK w rozpoznawaniu śluzowiaka zatok przynosowych.
Value of CT Imaging in Diagnosing Mucocele of Pranasal Sinuses.

30. Z. Borzęcki, F. Woźniak, Z. Święs: Assessment of Long-lasting Effect of Various Doses of Sinequan on Microstructure of the Male Gonad of the White Rat.
Ocena przewlekłego wpływu różnych dawek Sinequanu na mikrostrukturę gonady męskiej szczura białego.

Adresse:

UNIWERSYTET MARII CURIE-SKŁODOWSKIEJ
BIURO WYDAWNICTW
Plac Marii
Curie-Skłodowskiej 5 20-031 LUBLIN POLOGNE

Cena zł 10.200,–