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BIOCHEMICAL AND PHARMACODYNAMIC ARGUMENTS TO SUPPORT PROCAINE IMAO "B" TYPE ACTION

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Summary. The improvement of neuro-psychic functions and particularly of depressive states in the procaine-treated aged was pointed out for the first time by Ana Aslan. Subsequently numerous clinical and experimental researches have demonstrated the action of this substance on the central nervous system. The psychological and electroencephalographic investigations have completed the clinical observations.

In procaine biochemical mechanism of action an important part is played by its ability to inhibit monoaminoxidase. Based on a series of pharmacodynamic tests, the authors tried to define the type of inhibition. Researches in rats pointed out that procaine antagonises the worsening of conditioned behavior, intensifies and prolongs hyperthermia, delays reserpine-induced hypothermia and does not prevent reserpine-induced palpebral ptosis. These data suggest a B-MAO selective inhibitory effect. Contrary to the classical MAO inhibitors, procaine-based products are well tolerated by the aged patients.

Relatively recent data published in the field literature point out procaine depressive action.

Lüth [1] showed that Parhon and Aslan were the first scientists who mentioned procaine action on psychic states, in other words, the psychic effect of procaine. Aslan and coll. evidenced procaine eutrophic effect on the nervous system, manifest as improved memory, concentration and perception abilities, diminished insomnia and fatigue, increased desire to enjoy living and working, increased ability to adapt to the environment. A favourable influence upon the depressive psychical states of the aged was especially noticed [2–5]. Bucci and Saunders' study [6] conducted on 24 chronic psychotic patients followed up from 2 to 25 years pointed out that procaine (100 mg procaine hydrochloride 3 times per week for 6 months) improved depressive states and diminished psychotic symptoms associated with schizophrenia. In an experiment done on 32 hospitalized patients and 20 outpatients, aged 72–81, with senile and arteriosclerotic psychoses, Kral and coll. [7] found a procaine-induced transient improvement of depression accompanied by increased physical and psychical activity [9]. A double-blind study carried out by Smigel and coll. [8] on 60 patients with arthritis, nervous and senile disturbances pointed out significant improvements in 25 out of 29 patients treated with procaine for 5 months, whereas in the control group, the condition improved in only 9 out of 21 patients. The authors noticed procaine favourable effect particularly in patients with chronic nervous disturbances and less in those with chronic cerebral syndrome.

Lewicki and coll. [9] reported improvements in memory, thinking and associations in 75% of the aged subjected to the long-term treatment with Gerovital H_3 [10]. In a comparative study on 42 patients aged 73—80 suffering from arteriosclerotic senile dementia [38], alcoholic dementia with Korsakov syndrome [3], alcoholic senile dementia [1], Schneeberger [10] found that the procaine-based chronic treatment resulted in obvious improvements in one third of the treated cases. Cheerfulness, behavioral disturbances, morbidity improved. If prior to the treatment the patients had been completely indifferent to the environment, subsequently they reached an almost normal psychic level. None of these changes was noticed in the controls (quoted by [11]). Siggelkov (1966) subjected to the chronic treatment 74-year-old patients with cerebral atherosclerosis and deficient cerebral arterial circulation and found in 51.5% the alleviation of symptoms, expressing a better general reactivity of the organism: improvement of depressive states, irritability, motor and sensorial disorders, psychic deficiencies and mental confusion. Cohen and Ditman [12] carried out an open study on 41 patients: 17 normal, 17 psychiatric and 7 depressive patients with or without anxiety; they found that the administration of 100—200 mg Gerovital H_3 daily, 3 times a week for 4 weeks, resulted in the improvement of the general condition, energy, libido, motivation and somatic disturbances. S. Mora [13] noted important EEG differences between 139 subjects, mean age 66 and younger subjects, mean age 27. Combinations of alpha and beta waves or prevalence of beta waves were found in 67.7% of the aged subjects. The corresponding percentage in the younger subjects was 32%. Improved EEG tracings were pointed out in one third of the treated cases, whereas no modification was noticed in subjects with beta waves prevalence. The author inferred a possible correlation between psychic reactivity and cerebral electric activity.

The EEG investigations carried out in 1962 by Aslan and coll. pointed out normal electric tracings with no abnormality induced by the luminous intermittent stimulation in 75% of the cases (mean age 85) subjected to a long-term treatment with Gerovital H_3; in controls (untreated subjects) normal tracings were revealed in only 20% of the cases [4, 5].

Research efforts were further directed toward pointing out procaine biochemical mechanisms of action on the central nervous system. In 1940, Philpot [14] showed that procaine induced *in vitro* the inhibition of rat and guinea pig liver monoamineoxidase (MAO) by 60—100%. In 1950, Aslan and Vrăbiescu also noticed the inhibition of MAO and showed that procaine i.v. injections resulted in increased arterial pressure as a reaction to epinephrine [5]. In experiments conducted *in vitro* on rat liver, brain and heart, Hrachovec [15] pointed out that Gerovital H_3 induced a stronger MAO inhibition than procaine (Table 1). MacFarlane and Besbris [16] found procaine to act as a rapid and reversible inhibitor of brain MAO, which was proved *in vitro* by the decrease in the catecholamine and kynuramine metabolisms. The authors found that a 50% inhibition of MAO, requires amounts over 1 mg/ml procaine. They used MAO from rat brain mitochondrial preparations or homogenates and much higher procaine concentrations than the maximum serum levels *in vivo*. Yau [17] found the optimum procaine concentration required by MAO inhibition *in vivo* to be 10^{-6} M. Such concentrations induce *in vitro* only a 20 to 40% inhibition of mouse brain MAO. The same author, injecting i.p. quite large procaine amounts (90—180 mg/kg body weight) found a weak, but significant increase in cerebral serotonine and no significant change in dopamine or norepinephrine, indicating the weak or inexistent MAO inhibition. The same

*Table 1**

The inhibitory effect of Procaine HCl on MAO in rat brain, liver, heart and platelets

In vitro experiments

Inhibitor	Concentration of inhibitor	Percent inhibition			
		Brain	Liver	Heart	Platelets
Procaine HCl	1.10^{-4} M	31.5±2.3	37.8±1.7	36.4±1.4	28.9±0.9
Procaine HCl	1.10^{-3} M	64.7±2.8	58.5±2.9	59.4±2.1	51.5±0.9
Gerovital H_3	1×10^{-4} M	51.4±2.6	45.7±2.9	46.8±1.4	41.6±1.0
Gerovital H_3	1×10^{-3} M	87.4±1.9	74.2±2.1	69.9±2.3	69.1±1.2

In vivo experiments

Inhibitor	Dose of inhibitor	Percent inhibition		
		Brain	Liver	Heart
2% Procaine HCl	1.0 ml	16.7±0.9	13.6±1.2	14.3±1.2
2% Procaine HCl	2.0 ml	24.9±1.2	34.8±0.5	28.1±1.0
Gerovital H_3	1.0 ml	27.3±0.9	24.7±0.9	26.9±1.2
Gerovital H_3	2.0 ml	39.4±0.6	43.8±1.0	45.1±0.8

* (after Hrachovec J. P., The Physiologist, 1973, **15**, 3)

author did not find any significant changes in the above-mentioned amines subsequent to the chronic administration of 90 mg/kg body weight. Some MAO preparations were also found to be more sensitive to procaine.

These results called for an attempt to explain procaine antidepressive action, at least in part based on its IMAO effect which seems justified at present by the innumerable data accumulated that reveal the role of MAO in the function of the nervous system. The following facts were pointed out: cerebral MAO activity reaches quite high values; there is an ununiform distribution of MAO through the different areas of the brain, the enzyme prevailing in the hypothalamus; the independent and differentiated enzyme formation is possible in different cerebral structures; there is a significant variation of MAO concentration through the nervous tissue in the course of the ontogenetic evolution; there is a substantial change in the cerebral enzyme concentration and specific activity with advanced ages and certain psychic diseases.

Based on the use of specific inhibitors (chlorgyline and deprenyl), two major types of enzymatic activity have been pointed out: A-MAO and B-MAO. A-AMO, also called 5-hydroxytriptaminoxidase acts on 5-HT-norepinephrine (preferentially), thyramine, dopamine and is selectively inhibited by chlorgyline, which has a marked affinity for 5-HT-minergic receptors, the blockage of which is incomplete.

B-MAO, also called phenylethylaminoxidase acts on phenylethylamine bensylamine (preferentially) thyramine, dopamine and is selectively inhibited by deprenyl (isomer). Gascoigne and coll.'s histochemical investigations ints rat brain [18] pointed out a MAO variant which metabolized 5-HT, but was not sensitive to chlorgyline; they considered it a new form of enzyme, named C-MAO. Recent data have suggested the existence of a new MAO, with dopamine as favourite substratum [19].

As far as procaine inhibitory effect is concerned, although Young and coll. postulated the existence of certain MAO types particularly sensitive to procaine inhibitory effect, no mention has been made of the type of inhibition.

Starting from the fact that procaine IMAO effect is pointed out particularly *in vitro* and because of the lack of data in the field literature referring to the type of procaine IMAO action, we decided to use a battery of pharmacodynamic tests in order to check procaine antidepressive effect and point out *in vivo* procaine IMAO effect for specifying the type of inhibition. Procaine antidepressive effect was studied by means of the test of reserpine-induced conditioned behavioral changes in rats; IMAO effects were investigated by means of pharmacodynamic tests indirectly pointing out actions such as those attributed to MAO inhibitors (tests of hypothermic action, prevention of reserpine-induced hyperthermia and palpebral ptosis in rats).

Our experimental results [20] showed that 5 mg/kg body weight procaine antagonized reserpine-induced worsening of the conditioned behaviour in rats pointing out an antidepressive action comparable to a certain extent with that specific to tricyclic antidepressives (Table 2); on the other hand the same procaine amount strengthens and prolongs hyperthermia, delays reserpine-induced hypothermia and does not hamper reserpine-induced palpebral ptosis; these results support the clinical data which grant procaine IMAO properties and suggest the B type MAO inhibiting action (Tables 3 and 4).

The data are numerous which support procaine antidepressive effect due to MAO inhibition. The therapeutic use of specific MAO inhibitors in psychic diseases [21] characterized by changes in MAO concentration (schizophrenia, Parkinson's disease, certain forms of depression) as well as in aging, which results in the alleviation of psychiatric symptoms and age-related nervous disturbances, also support procaine antidepressive action through its IMAO effect.

CONCLUSIONS

Procaine antidepressive properties are all the more interesting as the substance is better tolerated by aged and ill persons than the classical MAO inhibitors which are potential therapeutic risk factors, because of their much too stronger effect, unspecific character and inhibition of a large spectrum of drug metabolized enzymes.

The study of procaine antidepressive action should be a current research priority because of its fundamental implications and clinical applicability. As we have already mentioned, our results suggest that procaine induces the selective inhibition of B-MAO.

Table 2

Influence of procaine and reserpine on the conditioned behaviour of rats

Lot of experiment	Period 0—30 minutes				Period 0—60 minutes			
	Electrical stimuli	Differences	Tactile stimuli	Differences	Electrical stimuli	Differences	Tactile stimuli	Differences
			Group A*					
Control group	0		0		0		0	
Procaine 5 mg/kg.b.wt. i.p. 5 days	0		0		0		0	
Reserpine 2 mg/kg.b.wt. i.m. (24 hrs before recording)	0		106.2 (±17.04)		0		251.1 (±38.22)	
Procaine 5 mg/kg.b.wt. i.p. 5 days + reserpine 2 mg/kg.b.wt. i.m. (24 hrs before recording)	0		30 (±23.43)	−76.2 p∼0.02	0		73.3 (57.04)	−177.8 p∼0.02
			Group B**					
Control group	0		87.6 (±34.38)		36. (±34.61)		213.6 (±85.35)	−100.8
Procaine 5 mg/kg.b.wt. i.p. 5 days	0		48 (±36.54)	−39.6 p∼0.5	88.8 (±73.62)	52.8 p∼0.5	112.8 (±84.64)	p∼0.4
Reserpine 2 mg/kg.b.wt. i.m. (24 hrs before recording)	43.2 (±40.37)		160.8 (±22.05)		76.8 (±68.34)		418.8 (±87.37)	
Procaine 5 mg/kg.b.wt. i.p. 5 days + reserpine 2 mg/kg.b.wt. i.p. (24 hrs before recording)	15.6 (±9.99)	−27.6 p∼0.6	102 (±53.40)	−58.8 p∼0.3	50.4 (±36.32)	−26.4 p∼0.7	196 (±100.48)	−222.8 p∼0.1

The mean number of tactile and electrical stimuli is given in relation to the conditioned reflex of the 1st and 2nd order, respectively (the figures in parentheses indicate standard error; significance of differences was calculated by the "t" test).

* 21 rats, which easily developed an optimal behaviour (well-balanced)

** 15 rats, incapable of having a proper conditioned behaviour (agitated, aggressive).

Table 3

Effect of procaine and reserpine, administered separately or in association, on the rectal temperature of rats

	$T°_m$	30′	60′	90′	120′	190′	240′
Single procaine dose 5 mg/kg.b.wt., i.p.	37.5(±0.24)	36.8(±0.86) dif. =0.7 p <0.005	36.9(±0.45) dif. = 0.6 p >0.025	37.26(±0.44) dif. =0.3 p = N	37.49(±0.11) dif. =0.01 p = N		
Single reserpine dose 2 mg/kg.b.wt., i.m.	37(±0.20)	37.78*(±0.55) dif. = 0.78 p >0.001	37.92**(±0.59) dif. =0.92 p >0.001		36.4(±0.35) dif. =0.6 p >0.05		
Single reserpine dose after procaine	37.53(±0.48)	38.07±0.92 dif. = 0.6 p <0.005	38.25±0.67 dif. =0.72 p <0.005		38±0.55 dif. =0.47 p <0.005		36±0.8 dif. =0.63 p = N
Reserpine after chronical administration of procaine (5 days).	37.7±0.61	37.4±0.78 dif. = 0.3 p = N	37.6±0.52 dif. =0.1 p = N	37.6±0.12 dif. =0.3 p = N	37.4±0.54 dif. =0.3 p = N	37.2±0.63 dif. =0.5 p = N	36.1±1.1 dif. =1 p <0.05

The figures in parentheses indicate standard error; significance of differences (p) was calculated by the "t" test.
* Mean value of t° in the 7 rats which presented hyperthermia.
** Mean value of t° in the 5 rats which presented hyperthermia.

Table 4

Influence of procaine on palpebral ptosis induced by reserpine in rats (3 lots of ten rats each)

	Total scores			Modification of reserpine effect on ptosis (%)		
	30′	60′	90′	30′	60′	90′
Reserpine 2 mg/kg.b.wt., i.m.	15	25	30			
Reserpine 20′ after a single procaine dose—5 mg/kg.b.wt., i.p.	11	25	32	−9	0	+6
Reserpine 20′ after the last dose of procaine administered chronically (5 days).	11	27	32	−9	+8	+6

Differences are statistically non-significant.

Résumé. L'amélioration des fonctions neuro-psychiques et, spécialement, des états dépressifs chez les personnes âgées traitées au Gérovital H_3, a été mise en évidence pour la première fois par Ana Aslan. Ultérieurement, de nombreuses recherches cliniques et expérimentales ont démontré l'action de cette substance sur le système nerveux central. Les investigations psychologiques et électroencéphalographiques ont complété les observations cliniques. Du point de vue biochimique, dans le mécanisme d'action de la procaïne un rôle important est détenu par sa capacité d'inhibition de la monoaminoxydase.

Les auteurs ont essayé de définir le type d'inhibition en utilisant une série de tests pharmacodynamiques. Les recherches sur les rats ont révélé que la procaïne est antagoniste à l'aggravation du comportement et qu'elle intensifie et prolonge l'hyperthermie, retarde l'hyperthermie induite par la réserpine et ne prévient pas la ptose palpébrale induite par la réserpine. Ces données suggèrent un effet inhibiteur du B-MAO. Contrairement aux inhibiteurs classiques MAO, les produits à base de procaïne sont bien tolérés par les patients âgés.

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