AKADEMIJA NAUKA I UMJETNOSTI BOSNE I HERCEGOVINE

RADOVI

KNJIGA LI

ODJELJENJE MEDICINSKIH NAUKA

Knjiga 18.

Urednik
PAVEL STERN,
redovni član Akademije nauka i umjetnosti
Bosne i Hercegovine

SARAJEVO
1974.

PAVEL STERN and JOSIP BORAS*

PHARMACOLOGICAL ANALYSIS OF HISTAMINE FUNCTION IN CENTRAL NERVOUS SYSTEM**

(Primljeno na sjednici Odjeljenja medicinskih nauka 16. X 1972. god.)

It has been known for a long time that histamine (H) is present in CNS (Clark and Ungar, 1964) but his central function is still obscure. There are controversial opinions on the transmitting role of H although it is known that synaptosomes in CNS contains H (Michaelson and Dowe, 1963). The distribution of this biogenic amine in CNS has been proved both in animals (Adam, 1961; Adam and Hye, 1966; Cavallito et al. 1970) and humans (McGeer, 1964). The highest amount of H has been found in the hypothalamic region (Adam, 1961, Adam and Hye, 1966). The enzymes which produce (histidin-decarboxylase) (Schwartz et al. 1970) or inacitivate (imidazole-N-methyl-transferaze (IMT)/(Kuhar et al. 1971) H have been found in the brain. When substances which can inhibit IMT or histidin-decarboxylase were discovered, a new experimental approach to the study of H in CNS became available. The problem is interesting from the physiological and pathophysiological point of view since it is known, for example, that antihistaminic agents have a good effect on motion-sickness (Brand and Perry, 1966). It is also known that shizophrenic patients are insensitive to H (Leblanc and Lemieux, 1961, Stern, 1956). At the same time, there is a hypothesis that shizophrenia could be an autoimune disease; in that case the role of H could be more important (Pfeiffer et al. 1970; Heath and Krupp, 1967). We have mentioned only these two facts to illustrate the importance of the further investigations of the role of H in this field.

A few years ago, we were interested to see the effect of the elevated H in CNS. IMT was inhibited by $CuCl_2$ (Stern, 1968). We showed

* Adress: Department of Pharmacology and Toxicology, Medical Faculty, 71000 Sarajevo.

** This work was supported by the Republic Fond for Scientific Research of Bosnia and Herzegovinia.

This paper was presented at the Fifth International Congress on Pharmacology, July 23—28, 1972, san Francisko.

that H potentiated the effect of drugs which increased acetylcholine in the brain and vice versa.

For example, the increased brain H potentiates oxotremorine und arecoline induced tremor, but not the tremor caused by LSD and prolonged hexobarbitone activity. The former tremorogenic agent increases the brain acetylcholine level and the latter does not. The increased level of H in CNS abolished pentazol-induced convulsions. It is interesting that tri-fluoperazine has no effect on the tremor, while chlorpromasine increases H in CNS and inhibits tremorine tremor. It should be mentioned that oxotremorine, arecoline and hexobarbitone increase the brain acetylcholine level without inhibition of cholinesterase, contrary to physostigmine and armine tremor. The increase of H in CNS has no effect on this kind of tremor. It was, however, more interesting to determine the function of different agents when brain H was decreased. Medina et al. (1969) have found that decaborane (D) significantly decreases H in CNS. This finding was confirmed by Schayer and Reily (1971). The effect of D is due to the inhibition of histidin-decarboxylase which causes the decrease of some other biogenic amines such as serotonin, noradrenaline and dopamine. Menon et al. (1971) have found that 4-thiazolyl-methoxyamine (TAM) also decreases H in CNS, while the level of other biogenic amines remains unchanged, except for a moderate drop of dopamine level.

This paper describes the influence of D (Stern, 1971) and TAM on rotatory nystagmus and some other pharmacological responses (Stern, 1971)*.

Before describing the methods and results, it is necessary to point out that in normal animals the decrease of brain H, caused by D or TAM, did not produce any observable change in the behaviour or appearance of some clinical symptoms. The animals drank, ate and walked normally etc.

METHODS

A. Rotatory nystagmus

D was diluted in olive oil and administered in doses of 15 mg/kg i.m., 24 hours before the experiments, when the concentration of H in CNS reached its lowest level. Wistar rats of both sexes were used in the experiments. Nystagmus was developed on a rotating disc (20 rotations in 17 seconds, radius 30 cm) (Stern, 1971). TAM was dissolved in distilled water and injected i.p. at the dose od 1000 mg/kg. Nystagmus was induced 72 hours after the drug injection. Rats of the same strain, but with the signs of ottitis media (side positions of the head) were also tested, because the position of the head is due to labyrinth excitation.

---

* The amount of TAM available to us, was not sufficient for all experiments. For these reasons, we could not make all experiments with TAM, so we made some with D, as usually. We acknowledge Dr Clark (Sepulveda) for TAM.

Since D also decreases other biogenic amines, it was necessary to treat animals with Cl-amphetamine in order to decrease serotonin, with alfa-methyl-methatyrosine to decrease dopamine and noradrenaline and 4-naphtylvinilpiridine to inhibit cholin-acetylase (S t e r n, 1971).

B. Aggressiveness

Male mice (Institute »Pasteur«, Novi Sad strain) weighting 20 g, were isolated for 3 weeks (Y e n et al., 1959). Only the animals which show aggressiveness toward other mice, when put in their cage, were injected the usual amount of D or TAM.

C. Allergic encephalomyelitis

The classic procedure for the induction of allergic encephalomyelitis was used (W a k s m a n, 1959). The animals were injected the suspension of Freund's adjuvans and the homogenate of the spinal cord. The effect of D in such condition was tested both preventively and therapeutically (These experiments are part of M. Sc. thesis of J. B o r a s).

D. Determination of D on gnawing of rats (an equivalent of vomit-caused by apomorphine) (J a n s s e n et al., 1960).

Rats were injected apomorphine (15 mg/kg i. p.) and 24 hours before they had received D.

E. Testing of agents which act centrally and cause classical neurological symptoms in mice

All animals were injected D. Twenty four hours later, the animals were injected the drugs listed on the table. Some of the animals were investigated 72 hours after TAM had been applied. We were trying to find out if the symptoms were potentiated, weaker or unchanged.

RESULTS

It can be seen from the table I that D decreases singnificantly the time of nystagmus of rats. The decrease of serotonine, nor-adrenaline and acetylcholine has no effect on nystagmus. It can be seen that TAM abolishes rotatory nystagmus, the same as D. Rats with ottitis media keep their head straight after receiving D.

The aggressive mice did not change their behaviour after the application of D or TAM. The results with the allergic encephalomyelitis are very interesting. It can be seen from the table, that D markedly prevents the evolution of the allergic encephalomyelitis and act favourably in therapy.

Apomorphine induced symptoms which represent an aquivalent of vomiting, were inhibited by D as well.

The table IV shows, that the agents which we used, following the application of D, had the opposite affect to that caused by the increase of brain H.

It is interesting to see Table V. The effect of the increase or decrease of H upon various drugs can be noted. The decrease was induced by D or TAM and the increase by $CuCl_2$. It is evident, that the effects are not always identical if we decrease H by using D or TAM.

DISCUSSION

We presume that our observation that D shortens or even eliminates nystagmus is very important, because antihistaminics are more effective against motion-sickness (Brand and Perry, 1966). It is interesting that the decrease of acetylcholine in CNS caused by a specific inhibitor of cholinacetylase (4-/1-naphtylvinil/piridine (Cavallito et al., 1970) had no effect. We mention this fact here, because we know that many anticholinergics give positive results in the treatment of the different sorts of motion-sickness (Brand and Perry, 1966). We have already shown that the increase of H in CNS prolongs nystagmus. We have therefore concluded with certainity that H is more important in the pathophysiology of nystagmus than acetylcholine. We lay stress on this fact particularly because we were able to show that H promotes acetylcholine effects in CNS.

The decrease of H in CNS gives the expected results. It is well known that antihistaminic agents act, not only on rotatory nystagmus, but also on vomiting (Brand and Perry, 1966).

By the decrease of H in CNS, these symptoms also were inhibited. The results with the allergic encephalomyelitis require special attention. It is known that the allergic reactions in the brain, as far as H is considered, are similar to the allergic reactions in different tissues (Mihajlović et al., 1964).

We have shown this in the allergic eczema of the skin (Stern, 1959) and in inflamation, using the method of granuloma pouch (Stern et al., 1956). Therefore, the question arises whether the improvement of symptoms of the allergic encephalomyelitis, which we have noticed, is the consequence of the H decrease in the brain. It can be seen that H weaknes the effect of the drugs that increase acetylcholine in CNS e. g. oxotremorine-tremor. D has no effect on the static tremor caused by LSD because acetylcholine is not changed in CNS. On the other side, D strenghtens convulsions caused by pentazol (Stern, 1971), which leads to a drecrease of acetylcholine in CNS. This means, that we have got a practicaly reversed situation in comparison with the one when H was increased in CNS after the administration to $CuCl_2$. We can therefore say with great probability that the decrease of H in CNS also manifested in some drugs through the function of acetylcholine. It is of interest to mention that Campos and Jurupe (1970) have come to similar conclusions about the relation between H and acetylcholine in CNS. But, there are cases when there is no shift in acetylcholine level in CNS, or only a very slight, e. g. after the use of nicotine or electrochock, or L-methionin-sulfoxine, but the effects

are stronger in spite of all (S t e r n, 1971). It is of particular interest that D deepens morphine analgesia, although morphine, as we know, increases acetylcholine in CNS (S t e r n, 1968). It was therefore expected that D would diminish analgesia.

In our previous experiment, we mentioned that the increase of H in CNS also increases the intensity of analgesia, like the decrease of H in CNS. It is likely that there is no correlation between acetylcholine in CNS and the perception of pain. The decrease of H in CNS had no effect on pain reception in normal mice.

We have mentioned before, that D is the inhibitor of L-amino--acid-oxidase and that it leads to the decrease of serotonine as well as of noradrenaline (M e d i n a et al., 1969). We mention this because we think that the results we got in this analysis cannot be explained only by the decrease of H in CNS.

It is probable that the lowered level of serotonine and noradrenaline also played a role in the reactions mentioned before. However, this remark is not of great value for the analysis of the effect of D on nystagmus. In this experiment we have excluded the decrease of serotonine and noradrenaline caused by its specific depletors (S t e r n, 1971). It can be concluded with great probability that the effect of D on nystagmus is mediated by the decrease of H in CNS.

SUMMARY

The decrease of histamine in central nervous system supresses rotatory nystagmus, vomiting and diminisches the intensity of the allergic encephalomyelitis. We also showed that the decrease of histamine induced the opposite affect to that induced by histamine increase, when some agents which cause brain acetlycholine increase or decrease were applied.

P. STERN i J. BORAS

FARMAKOLOŠKA ANALIZA FUNKCIJE HISTAMINA U CENTRALNOM NERVNOM SISTEMU

KRATAK SADRŽAJ

Smanjenje histamina u centralnom nervnom sistemu inhibira rotatorni nistagmus, povraćanje i smanjuje intenzitet alergičkog encefalomijelitisa. Isto tako je pokazano da smanjenje histamina izaziva obrnut efekat od onog kojeg izaziva povećanje kada se apliciraju neki spojevi koji izazivaju povećanje ili smanjenje acetilholina u mozgu.

REFERENCES

A d a m, H. M.: *Histamine in the central nervous system and hypophysis of the dog.* In »Regional Neurochemistry, ed. Kety, S.S., Elkes, J. pp 293—306. Pergamon Press, London, 1961.

A d a m, H. M., H y e, H. K. A.: *Concentration of histamine in different parts of brain and hypophysis of cat and its modification by drugs.* Br. J. Pharmac. Chemother. *29*, 137—152 (1966).

Brand, J. J.: Perry, W. L. M.: *Drugs used in motion sickness.* Pharmacological Reviews. *18,* 895—924 (1966).

Campos, H. A., Jurupe, H.: *Evidence for a cholinergic mechanism inducing histamine increase in the rat brain in vivo.* Experientia *26,* 746—747 (1970).

Cavallito, C. J., White, H. L., Yun, H. S., Foldes, F F.: *Inhibitors of choline acetyltransferase.* In: Heilbronn, Edith, Winter, A. *Drugs and cholinergic mechanisms in the CNS;* Research Institute of National Defence, Stockholm, 1970 p. 97—116.

Clark, G. W., Ungar, G.: *Histamine and the nervous system,* a Symposium. Federation Proceedings *23,* 1095—1116 (1964).

Heath, R. C., Krup, I. M.: *Schizophrenia as an immunologic disorder.* Arch. Gen .Psychiat. *16,* 1 (1967).

Janssen, P. A. J., Nimegeers, C. J. C., Jageneau, H. M.: *Apomorphine antagonism in rats.* Arzneimittelforsch. *10,* 1003—1005 (1960).

Kuhar, M. J., Taylor, K. M., Snyder, S. H.: *The subcellular localization of histamine and histamine methyltransferase in rat brain.* J. of Neurochemistry *18,* 1515—1527 (1971).

Leblanc, J., Lemieux, L: *Histamine and mental disease.* Medicina experimentalis *4,* 214—222 (1961).

McGeer, P. L.: *The distribution of histamine in cat and human brain.* Comparative Neurochemistry. Proceedings of the 5th International Neurochemistry Symposium, Austrija June, 1962, Peargamon Press, Oxford, London, 1964, 387—391.

Medina, M. A., Landez. J. H., Forster, L. L.: *Inhibition of tissue histamine formation by decaborane.* J. Pharmac. exper. Therapeut. *169,* 132—137 (1969).

Menon, M. K., Clark. W G., Auers, D.: *Effect of thiazol-4-ylmethoxyamine, a new inhibitor of histamine biosynthesis on brain histamine, monoamine and behavior.* Life Sciences *10,* Part I, 1097—1109 (1971).

Michaelson, I. A., Dowe, G.: *The subcellular distribution of histamine in brain tissue.* Biochem. Pharmacol. *12,* 949—956 (1963).

Mihajlović, Lj. Janković, B., Beleslin, B., Mitrović, K. Kožalić, Lj.: *Effect of intraventriculary injected anti-cerebral antibodies on the histamine-like substance and potassium content of various regions of brain of the cat.* Nature *203,* 763—764 (1964).

Pfeiffer, C. C., Iliev, V., Goldstein, L., Jenney Elisabeth, H., Schultz, R.: *Blood histamine, polyamines and the schizophrenias computer correlations of the low and high blood histamine types.* Res. Comm. Chem Pathol. Pharmacol. *1,* 247—265 (1970).

Schayer, R. W., Reilly Margaret, A.: J. *Pharmac. exper.* Therapeut. *177,* 177—180 (1971).

Schwartz, J C., Lampart, C., Rose, C.: *Properties and regional distribution of histidine decarboxylase in rat brain.* J. of Neurochemistry *17,* 1527—1534 (1970).

Stern, P.: *Die Bedeutung des Histamins in der Entstehung des Ekzems.* Arch. int. Pharmacodyn *105,* 279—284 (1956).

Stern, P., Nikulin, A., Misirlija, A., Ciglar, M.: *Histamin im Entzündungsprozess.* Arch. exper. Path. u. Pharmakol. *227,* 522—527 (1956).

Stern, P., Huković, S., Mađarek, Ž. Karabaić, S.: *Histamingehalt im Blute von Schizophrenikern.* Arch. int. Pharmacodyn. *109,* 294—299 (1957).

Stern, P.: *Beitrag zur Wirkungsweise des Histamins im Zentralnervensystem.* Wien. klin. Wschr. *80,* 181—185 (1968).

Stern, P.: *Farmakološka analiza uloge histamina u centralnom nervnom sistemu.* Acta Pharm. Jug. *21,* 171—174 (1971).

Waksman, B. H.: *Allergic encephalomyelitis in rats and rabbits pretreated with nervous tissue.* J. Neuropathology and Experimental Neurology *19*, 397—417 (1959).

Yen, C. Y., Stanger, L. R., Millman, N.: *Ataractic suppression of isolation-induced aggressive behaviour.* Arch. int. Pharmacodyn. *123*, 179—185 (1959).

Table I

EFFECT OF VARIOUS DRUGS ON ROTATORY NYSTAGMUS

Substance	Dose (mg/kg)	Duration of nystagmus in seconds (± SE-N)	p
Controls	—	9.00 (± 0.87—12)	
Decaborane	15 i.m.	1.67 (± 0.43—12)	< 0.001
Chloramphetamine	10 i.p.	9.75 (±0.27—12)	> 0.05
— methyltyrosine	100 i.p.	9.91 (± 0,24—12)	> 0.05
4-(1-naphthylvinil)-pyridine	8 i.v.	10.18 (± 0.12—12)	> 0.05
Controls	—	9.50 (± 0.44— 6)	
TAM	100 i.p.	2.16 (± 0,28— 6)	< 0.01

Table II

PREVENTIVE EFFECT OF DECABORANE ON OCCURRENCE OF SYMPTOMS (PARESIS, PARALYSIS), OF EXPERIMENTAL ALLERGIC ENCEPHALOMYELITIS (EAE) IN RATS

SYMPTOMS OF EAE	TREATED GROUP (Decaborane 15 mg/kg i. m.)		CONTROL GROUP (Oleum Olivae 0,1 ml/100 g i. m.)		p
	Number of animals	% occurrence of symptoms ± SE	Number of animals	% occurrence of symptoms ± SE	
WITHOUT SYMPTOMS	50	90±0,042	50	6±0,033	<0,001
PARESIS	50	8±0,037	50	14±0,048	>0,05
PARALYSIS	50	0±0,000	50	70±0,064	<0,001
LETHAL END	50	2±0,019	50	10±0,042	>0,05

Table III

THERAPEUTIC EFFECT OF DECABORANE ON THE SYMPTOMS (PARESIS, PARALYSIS) OF EXPERIMENTAL ALLERGIC ENCEPHALOMYELITIS (EAE) IN RATS

SYMPTOMS OF E A E	TREATED GROUP (Decaborane 15 mg/kg i. m.)		CONTROL GROUP (Oleum Olivae 0,1ml/100 g) i. m.)		p
	Number of animals	% of animals with evident therapeutic effect ± SE	Number of animals	% of animals with evident therapeutic effect ± SE	
Paresis	19	36,8±0,111	12	0±0,000	<0,01
Paralysis	21	23,8±0,093	28	0±0,000	<0,05
The total number of animals	40	30,0±0,022	40	0±0,000	<0,01

Table IV

BEHAVIOUR OF THE RATS	Control 5 mg/kg	Treated animal 5 mg/kg + Decaborane	Control 10 mg/kg	Treated animal 10 mg/kg + Decaborane
The animal is quiet	0%	20%	0%	0%
The animal moves its gnaws horizontaly	0%	70%	0%	10%
The animal bites the Paper on its edges	80%	10%	0%	90%
The animal tears paper into pieces	20%	0%	100%	0%

Table V

	Decrease H in CNS		Increase H in CNS
	D	TAM	
Oxotremorine	—	0	+
LSD	0	+	0
Morphine	+	+	+
Chlorpromasine	+	+	+
Pentasol	+	+	—
Strychnine	+	+	0
Amphetamine	—	—	—
Hexobarbiton	—	0	+
Nystagmus	—	—	+
Aggressive mice	0	0	0

0 = without effect
— = effect weaker
+ = effect stronger