Clinica Medica,
Facoltà di Medicina,
Università Cattolica del Sacre Cuore,
Rome,
Italy

P. U. CARBONIN
G. GAMBASSI

REFERENCES

1. B. CHANCE and G. R. WILLIAMS, *Adv. Enzymol.* **17**, 65 (1956).
2. J. L. WEBB, *Enzyme Metabol. Inhibitors* **1**, 518 (1963).
3. P. U. CARBONIN, A. PIFFANELLI, P. ZECCHI, A. V. GRECO and G. GAMBASSI, *Aggiorn. Mal. Infez.* in press.
4. J. L. WEBB, *Br. J. Pharmac.* **5**, 87 (1950).
5. A. E. REIF and R. POTTER *Cancer Res.* **13**, 49 (1953).
6. G. H. HOGEBOOM, *Methods in Enzymology*. Vol. 1, Academic Press, New York (1955).
7. D. M. GREENBERG, *J. biol. Chem.* **82**, 545 (1929).
8. W. W. ACKERMAN and V. R. POTTER, *Proc. Soc. exp. Biol. Med.* **72**, 1 (1949).
9. K. AHMED, H. G. SCHNEIDER and F. M. STRONG, *Archs Biochem.* **28**, 281 (1950).

Biochemical Pharmacology, Vol. 16, pp. 1132–1134. Pergamon Press Ltd. 1967. Printed in Great Britain

Effect of physostigmine on the level of brain biogenic amines in rats and rabbits

(*Received* 2 *November* 1966; *accepted* 20 *December* 1966)

PHYSOSTIGMINE (eserine) is a reversible inhibitor of cholinesterase and potentiates peripheral as well as central effects of acetylcholine. In both regions its pharmacological actions are different in many ways from other parasympathomimetic drugs such as metacholine or carbamylcholine. The necessity of interpreting the actions of physostigmine solely on the basis of its ability to inhibit cholinesterase has been removed by a series of papers by Varagić and co-workers.[1–3] They concluded that one action of physostigmine is due to a central stimulation of adrenergic nervous elements which is reflected in an elevation of blood pressure after injection of physostigmine to anesthetized rats. They find that acute electrolytic lesions in various parts of the hypothalamus cause a depression or complete abolition of the hypertensive effect of physostigmine. In addition, the blood pressure responses are antagonized by sympatholytic drugs. They feel that a central activation of adrenergic elements take place at a level no lower than the medulla oblongata.

We therefore decided to investigate the effect of large doses of physostigmine on the brain biogenic amines, serotonin and norepinephrine, hoping to demonstrate a direct effect of this drug on these substances.

METHODS

Rabbits. Ten male New Zealand albino rabbits ranging in weight from 3·0 to 3·8 kg were used. They were divided into three groups: a control group of four animals and two experimental groups of three rabbits each. The experimental groups were given 0·3 mg physostigmine/kg (physostigmine sulfate, Merck) i.v. It was made up as a 0·3% solution in saline and administered slowly into the marginal ear vein during 2 min. The members of one group of rabbits were killed 30 min and the others at 4 hr after the injection of physostigmine. The control groups were given saline solution in the same volume. The animals were stunned by a blow on the neck and bled by opening the carotid arteries. The brain was rapidly extirpated, washed in saline, and frozen on solid carbon dioxide. The brain, less cerebellum, was divided into two parts—hemispheres and brain stem.

Rats. Twenty-six male, Wistar-strain rats ranging in weight from 360 to 400 g were divided into five groups: a control group (6 rats) and four experimental groups (5 rats each). All members of two experimental groups were given 1·5 mg physostigmine/kg in a single i.p. injection; the other two groups received a total each of 3·0 mg/kg divided into three injections of 1·0 mg/kg i.p. spaced 5 min apart. The control rats received saline and were killed 30 min after injection. The experimental animals were killed either 30 min or 4 hr after the last injection. The animals were decapitated and the whole brain rapidly removed, washed in saline, and frozen on solid carbon dioxide. The brain contents of serotonin and norepinephrine were determined on the same sample after homogenization and butanol extraction according to the procedure of Mead and Finger.[4] Norepinephrine was measured by the trihydroxyindole technique and serotonin by native fluorescence in strong hydrochloric acid.

RESULTS

Toxic signs and symptoms. The rabbits, shortly after the i.p. injection of physostigmine, exhibited a brief attack of muscular fasciculations or convulsions lasting 2 or 3 min. After that period the animals revealed no further toxic side effects, but the secretions of the salivary and bronchial glands were enhanced.

In preliminary toxicity tests all rats receiving physostigmine 1·5 mg/kg i.p. survived. However, in the experimental series, one rat given a 1·5 mg/kg dose died, as did three rats receiving 3·0 mg/kg. These animals were replaced by supplemental rats treated in the same manner. All rats receiving the drug exhibited tremors, mild convulsions, and chewing movements which began about 10 min after injection. Deaths, when they occurred, were observed 15–24 min after injection.

Levels of brain amines. The brain amine values are summarized in Tables 1 and 2. In rabbits, physostigmine had no effects on serotonin or norepinephrine. In rats the physostigmine at 1·5 mg/kg

TABLE 1. EFFECT OF INTRAVENOUS PHYSOSTIGMINE INJECTION ON BRAIN BIOGENIC AMINES IN RABBITS

Treatment and no. of rabbits	Serotonin (μg/g)		Norepinephrine (μg/g)	
	Hemispheres	Brain stem	Hemispheres	Brain stem
Control (4)	0·49 ± 0·07*	0·84 ± 0·09	0·32 ± 0·04	0·39 ± 0·01
Physostigmine (0·3 mg/kg, 30 min) (3)	0·46 ± 0·04	0·81 ± 0·04	0·27 ± 0·02	0·41 ± 0·06
Physostigmine (0·3 mg/kg, 4 hr) (3)	0·38 ± 0·07	0·78 ± 0·11	0·29 ± 0·03	0·41 ± 0·05

* Figures represent average ± standard deviation.

was equally ineffective. However, at the 3·0 mg/kg dose level, serotonin concentrations in the hemispheres were increased slightly 30 min after injection. The norepinephrine concentrations in hemispheres and brain stem were decreased both 30 min and 4 hr after the injections.

TABLE 2. EFFECT OF INTRAPERITONEAL PHYSOSTIGMINE INJECTION ON BRAIN BIOGENIC AMINES IN RATS

Treatment and no. of rats	Serotonin (μg/g)		Norepinephrine (μg/g)	
	Hemispheres	Brain stem	Hemispheres	Brain stem
Control (6)	0·55 ± 0·03*	0·93 ± 0·15	0·46 ± 0·04	0·77 ± 0·13
Physostigmine (1·5 mg/kg, 30 min) (5)	0·60 ± 0·05	0·98 ± 0·06	0·47 ± 0·09	0·80 ± 0·19
Physostigmine (1·5 mg, 4 hr) (5)	0·57 ± 0·06	1·02 ± 0·12	0·53 ± 0·07	0·90 ± 0·08
Physostigmine (3 mg, 30 min) (5)	0·64 ± 0·04†	0·93 ± 0·08	0·48 ± 0·04	0·59 ± 0·05†
Physostigmine (3 mg, 4 hr) (5)	0·54 ± 0·02	0·80 ± 0·10	0·47 ± 0·03	0·61 ± 0·06†

* Figures represent average ± standard deviation.
† Values differ significantly from control, at $P < 0·01$ level.

DISCUSSION

Our results show that convulsive and nearly lethal doses of physostigmine have only minimal effects on brain concentrations of serotonin and norepinephrine. The elevation of serotonin which we encountered has previously been observed by Costa and Himwich[5] in rabbits after brief convulsions induced by insulin. Similar results were obtained by Garattini and Valzelli[6] after electro-shock and pentylenetetrazol-induced convulsions in mice, rabbits, and dogs. The depletion of norepinephrine found after the highest dose of physostigmine may be ascribed to the effects of the convulsion or nonspecific stress-induced release of norepinephrine.[7, 8] It is of interest that nicotine, another drug with strong parasympathomimetic properties, also fails to reduce the content of brain amines in mice and guinea pigs.[9]

Physostigmine therefore does not appear to have a direct depleting or releasing action on brain amines, and the involvement of central adrenergic mechanisms must be sought either in terms of a nonspecific stressing effect or as a secondary activation of the adrenergic outflow from the brain.

Thudichum Psychiatric Research Laboratory,
Galesburg State Research Hospital,
Galesburg, Ill., U.S.A.

G. R. PSCHEIDT
Z. VOTAVA
H. E. HIMWICH

REFERENCES

1. V. VARAGIĆ and N. VOJVODIĆ, *Br. J. Pharmac.* **19**, 451 (1962).
2. M. KRSTIĆ and V. VARAGIĆ, *Br. J. Pharmac.* **24**, 132 (1965).
3. V. VARAGIĆ, M. KRSTIĆ and B. BELENSKIN, *Archs int. Pharmacodyn.* **162**, 162 (1966).
4. J. A. R. MEAD and K. F. FINGER, *Biochem. Pharmac.* **6**, 52 (1961).
5. E. COSTA and H. E. HIMWICH, Fourth International Congress of Biochemistry, Vol 3, *Biochemistry of the Central Nervous System*, p. 283. Pergamon Press, Oxford (1960).
6. S. GARATTINI and W. VALZELLI, *Psychotropic Drugs*, p. 428. Elsevier, Amsterdam (1957).
7. J. D. BARCHAS and D. X. FREEDMAN, *Biochem. Pharmac.* **12**, 1232 (1963).
8. E. W. MAYNERT and R. LEVI, *J. Pharmac. exp. Ther.* **143**, 90 (1964).
9. E. HANSSON, D. T. MASUOKA and W. G. CLARK, *Archs int. Pharmacodyn.* **149**, 153 (1964).