NOREPINEPHRINE SYNTHESIS AND TURNOVER IN THE BRAIN: ACCELERATION BY PHYSOSTIGMINE

TOMISLAV KAŽIĆ*†
Laboratory of Clinical Science, National Institute of Mental Health,
Bethesda, Maryland 20014, U.S.A.

CHOLINERGIC-adrenergic interactions have been shown to exist in peripheral organs supplied with dual autonomic innervation (KAŽIĆ, 1971; MUSCHOLL, 1970; PATON and VIZI, 1969). Both cholinergic and adrenergic neurons are well identified in the central nervous system (CNS), and acetylcholine (ACh) and norepinephrine (NE) are generally regarded as transmitter substances in the brain. Their close spatial distribution and a considerable body of experimental evidence indicate that an active interrelationship exists between the two parts of the autonomic nervous system in the brain.

After systemic administration, physostigmine, a reversible cholinesterase inhibitor, produces a hypertensive response in the rat, as do some other cholinesterase inhibitors which penetrate into the brain (VARAGIĆ, 1955, 1966). Because physostigmine has been shown to produce glycogenolysis in the brain and liver (MRŠULJA, 1968; VARAGIĆ *et al.*, 1967), increased neuronal activity in the preganglionic fibres of the cervical sympathetic chain (STAMENOVIĆ, 1955) and hypothermia (MYERS and YAKSH, 1968; VARAGIĆ *et al.*, 1971), it is believed that the drug produces a general noradrenergic activation. The sympathetic activation produced by physostigmine is of central origin (MRŠULJA *et al.*, 1968). Inhibition of cholinesterase enhances cholinergic activity which appears to trigger the central adrenergic mechanism responsible for the activation of the peripheral sympathetic nervous system (VARAGIĆ *et al.*, 1968). The present experiments were performed in order to examine the role of brain catecholamines on the sympathetic activating effect of physostigmine.

METHODS

Experiments have been carried out on male Sprague–Dawley rats (200–350 g body weight). Physostigmine (200 μg/kg, i.v.) was administered 5 min before a single rapid injection of L-tyrosine-^{14}C (44 μCi, i.v.; spec. activity 476 mCi/mmole), and 10 min later the rats were decapitated. All tissues were rapidly removed, brains carefully dissected into: brainstem, hypothalamus and forebrain (rest of brain), and all structures including spinal cords, hearts and salivary glands were quickly frozen in liquid nitrogen and stored at -70°C. Radioactive catecholamines were determined according to KOPIN (1972), while the endogenous NE was assayed using the technique of HAGGENDAL (1963).

6-Hydroxydopamine (6-OH-DA) was administered intracisternally (2 $\times$ 200 μg at 48 hr interval), and seven days after the second injection blood pressure responses

* International Postdoctoral Fellow, U.S. Public Health Service, Fellowship number 1 FO5 TW01827-01.

† Home Institute, Department of Pharmacology, Faculty of Medicine, 11.000 Belgrade, Yugoslavia.

to physostigmine were recorded under urethane anesthesia. A separate group of rats treated with 6-OH-DA intracisternally was used for determination of endogenous NE content in the brain, heart and salivary gland.

Atropine (2 mg/kg, i.p.), phenoxybenzamine (10 mg/kg, i.p.) and propranolol (10 mg/kg, i.p.) were administered 20–40 min before physostigmine (200 μg/kg, i.v.) in order to study their effects on the endogenous NE in the brain, and their interaction with the physostigmine-induced changes.

Tyrosine hydroxylase (TH) activity in the brain areas was determined after a prolonged administration of physostigmine (2×200 μg/kg, i.v., during 7 days) according to the technique of COYLE (1972).

RESULTS AND DISCUSSION

In rats under urethane anesthesia, the hypertensive response to physostigmine was shown to depend on the integrity of catecholaminergic neurons in the brain. After intracisternal pretreatment with 6-OH-DA this response was diminished by 40 per cent. Endogenous NE content in the brain was severely reduced (15–36 per cent of the control level). In conscious rats, treatment with physostigmine produced a rapid decrease in the endogenous NE content of the brainstem and hypothalamus, but not of the forebrain. A linear dose–response relationship was obtained with doses of physostigmine (100, 200 and 300 μg/kg, i.v.) and the reduction in the endogenous NE in the brain areas. Pharmacological analysis of this catecholamine-depleting action of physostigmine showed that it can be completely prevented with both atropine and propranolol. Atropine was also found not only to prevent the effect of physostigmine, but also to increase the endogenous NE content of the hypothalamus when given alone. Propranolol produced no change in the endogenous NE, however, pretreatment with this drug completely prevented the NE depleting action of physostigmine. Results obtained with phenoxybenzamine are inconclusive since this substance was found to produce a significant depletion of the endogenous NE stores in the brain, by itself.

Determination of the synthesis of radioactive NE from the labeled precursor, L-tyrosine-^{14}C, provided evidence that physostigmine, simultaneously with the elevation of the blood pressure in the periphery, produced an increase in the synthesis of the radioactive transmitter in the CNS. Synthesis of NE-^{14}C was enhanced in all brain areas, including those in which the endogenous NE levels had not been changed. Specific activity of the NE-^{14}C, considered as a measure of turnover, was also significantly increased.

TH assay of the enzyme activity in the brain areas performed after prolonged administration of physostigmine has shown that this substance is capable to produce a significant activation of the TH, particularly in the brainstem and hypothalamus, areas which seem to be main targets of the action of physostigmine. In addition, it should be pointed out that the repeated administration of physostigmine produced no change in the blood pressure levels of the treated animals, as compared to the saline-treated control group.

The present experiments support the view that the peripheral sympathetic activation, seen after systemic administration of physostigmine, involves activation of a central adrenergic mechanism. Administration of physostigmine results in increased synthesis and turnover of NE both in the CNS and in the peripheral sympathetic

nervous system. Increased activity of TH, rate-limiting enzyme in the catecholamine synthesis, has also been found in the hypothalamus and brainstem of the animals treated with physostigmine. Destruction of the brain catecholaminergic neurons with 6-OH-DA, blockade of the central muscarinic receptors with atropine and administration of a beta-adrenergic blocking agent, propranolol, were found to diminish or completely prevent the activating action of physostigmine on the adrenergic neurons in the brain.

It can be concluded, that the present investigation provides direct experimental evidence that an increase in synthesis, turnover and release of NE in the brain are the underlying mechanisms for the general adrenergic activation produced by physostigmine.

REFERENCES

COYLE J. T. (1972) *Biochem. Pharmacol.* **21,** 1935–1944.

HAGGENDAL J. (1963) *Acta physiol. Scand.* **57,** 242–254.

KAŽIĆ T. (1971) *Europ. J. Pharmacol.* **16,** 367–373.

KOPIN I. J. (1972) In: *The Thyroid and Biogenic Amines* (RALL and KOPIN I. J., Eds.) pp. 489–496, North-Holland, Amsterdam.

MRŠULJA B. B., TERZIĆ M. and VARAGIĆ V. M. (1968) *J. Neurochem.* **15,** 1329–1333.

MUSCHOLL E. (1970) In: *New Aspects of Storage and Release Mechanisms of Catecholamines.* (SCHÜMANN H. J. and KRONEBERG G., Eds.) pp. 168–186, Springer-Verlag, Berlin.

MYERS R. D. and YAKSH T. L. (1968) *Physiol. Behav.* **3,** 917–928.

PATON W. D. M. and VIZI E. S. (1969) *Br. J. Pharmac.* **35,** 10–28.

STAMENOVIĆ B. A. and VARAGIĆ V. M. (1970) *Neuropharmacology* **9,** 561–566.

VARAGIĆ V. M. (1955) *Br. J. Pharmac. Chemother.* **10,** 349–353.

VARAGIĆ V. M. and KRSTIĆ M. (1966) *Pharmacol. Rev.* **18,** 799–800.

VARAGIĆ V. M., KAŽIĆ T. and ROSIĆ N. (1968) *Yugoslav. Physiol. Pharmacol. Acta* **4,** (Suppl. 1), 113–120.

VARAGIĆ V. M., TERZIĆ M. and MRŠULJA B. B. (1967) *Arch. Pharmak. Exp. Path.* **258,** 229–237.

VARAGIĆ V. M., ŽUGIĆ M. and KAŽIĆ T. (1971) *Arch. Pharmak. Exp. Path.* **270,** 407–418.